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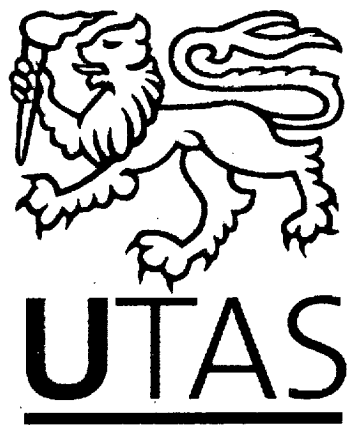
ESTROGEN TREATMENT FOR TALL STATURE IN ADOLESCENT GIRLS: SHORT- AND LONG-TERM EFFECTS ON THE BREAST

by

Helen L. Jordan, BSc (Hons), DipEd, GradDipEpid (UniMelb)

Submitted in fulfilment of the requirements for
the Degree of Doctor of Philosophy

University of Tasmania
September, 2010



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ABSTRACT

Adolescent tall girls have been treated with high-dose estrogens to reduce their final height for psychosocial reasons since the 1950s. Although the practice is uncommon now, a recent survey of US paediatric endocrinologists reported that 96 (23%) of 411 respondents had treated girls in the preceding 5 years[3]. Exposure to high-dose estrogen during the pubertal mammary development stage could have long-term effects on breast histology, function and disease. This study investigated the breast related side effects of treatment in a retrospective cohort study of Australian girls who, between 1953 and 1993, were assessed for tall stature in adolescence and either treated with high-dose estrogens (diethylstilbestrol or ethinyl estradiol) or untreated. Breast related side effects experienced during treatment; subsequent effects on lactation (breastfeeding initiation and duration), breast disease or investigations (e.g. breast biopsies); and mammographic density, a well established risk factor for breast cancer, were examined over two follow-up periods.

At the first follow-up (2002-2003) demographic information, details of assessment and treatment, and short-term side effects of treatment were collected from 371 treated and 409 untreated women via postal questionnaire. History of breast disease and investigations, pregnancy and breastfeeding history were collected by computer assisted telephone interview (CATI). Treatment and anthropometric variables in adolescence were obtained from medical records where available. At the second follow-up (2006-2007), 167 treated and 142 untreated women aged 40 years or older provided access to a recent mammogram from which dense area, percent density, non-dense area and total breast area were measured using a computer assisted thresholding method. Additional risk factor data were collected and/or updated in a second CATI.

Short-term effects of treatment reported by the women included breast lumps, galactorrhea, breast pain, dry cracked or bleeding nipples and increased pigmentation of the nipple and areolae. These effects were more frequently reported in women treated with diethylstilbestrol. Compared to untreated women, treated women were no more likely to have ever had a breast biopsy, breast surgery, or a diagnosis of breast cancer. There was no significant difference in the average duration of breastfeeding between treated and untreated women, and treated women were no more likely to not commence breastfeeding. Mammographic findings showed that treated women had a significantly lower mean dense area than untreated women but did not differ significantly in mean percent density, non-dense area or total breast area.

The short-term side effects of treatment reported by women in this study would have caused discomfort and possibly embarrassment in adolescence. However, this investigation provides some reassurance for women treated with high-dose estrogens for tall stature that treatment does not appear to affect their ability to lactate or increase their risk of having breast disease requiring a breast biopsy or surgery, and is unlikely to increase their risk of breast cancer through mechanisms related to mammographic density. The study also has broad implications for our understanding of the biology of breast development and for breast cancer research. It has shown us that exposure to sex hormones during adolescence can have a sustained effect on breast tissue as demonstrated by a reduction in mammographic dense tissue in adulthood.

CONTRIBUTION OF AUTHOR

My contribution to this PhD study included cleaning and analysing the data on breast health collected at follow-up 1 of the Tall Girls Study. I developed the follow-up 2 research question 'whether treatment with high-dose estrogens in adolescence is associated with mammographic density as an adult' and developed collaborations with Prof. John Hopper, Prof. Anne Kavanagh and Prof. Dorota Gertig to assist with equipment and training to undertake the study. I also contributed substantially to the development of the successful NHMRC project grant (403002; 2006) proposal to fund the second follow-up.

For the second follow-up of the study, I applied for ethics approval and developed the study recruitment materials including the information brochure, consent forms and invitation letters with guidance from my primary PhD supervisor. I recruited and worked closely with the study administrator Emma Stubbs and the interviewer Shirley Catchpole, whose positions were funded by the NHMRC project grant funds. Study administration involved setting up and maintaining a database, sending out the recruitment materials, following-up non-responders, organising telephone interviews with study participants and sending letters to BreastScreen services to request for the mammograms of women who provided their consent for them to be used in the study. I trained Shirley to perform the interviews and I undertook a number of the interviews that were scheduled after hours.

I modified the questionnaire that was derived from the Australian Twins and Sisters Breast Density Study (UniMelb) and worked with Tim Albion (IT specialist at Menzies Research Institute, UTAS) to translate the hard copy questionnaire into a computer assisted telephone interview tool. I modified the protocol for mammogram retrieval that was previously used by Anne Kavanagh and managed the retrieval, scanning and return of mammograms. I masked and read all films for density measurements and undertook all statistical analysis with guidance from Russell Thomson, a statistician at Menzies Research Institute.

I presented the findings of the short-term side effects on the breast at the Australasian Epidemiological Association Conference (Oct., 2005), the findings on the effects of treatment with high-dose estrogens on subsequent lactation at the Australasian Epidemiological Association Conference (Oct., 2006), and the mammographic density findings at The University of Melbourne (Dec., 2007, *Work in Progress*), the Menzies Research Institute, University of Tasmania (Sept., 2008), the International Endocrinology Association (Nov., 2008) and the American Association of Cancer Research (Nov., 2008).

I also contributed substantially to the conception, design and drafting of two research articles drawn from this PhD research 1) Jordan H. L., Bruinsma F. J., Thomson R. J., Amir L. H., Werther G.A. & Venn A. J. Adolescent exposure to high-dose estrogen and subsequent effects on lactation, *Reprod Toxicol* 2007; 24: 397–402. 2) Jordan H.L., Hopper J.L., Thomson R.J., Kavanagh A.M., Gertig D.M., Stone J. & Venn A.J. Influence of high-dose estrogen exposure during adolescence on mammographic density for age in adulthood. *Cancer Epidemol Biomarkers and Prev* 2010; 19:121–9.

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I also owe my deepest gratitude to Professor David Dunt, my co-supervisor, work colleague and mentor, for his insistent prodding of me to undertake a PhD in the first place, his continued encouragement and support throughout the process, and his guidance in the development of this thesis. His persistence and expertise has been valuable.

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This thesis would not have been possible without the financial assistance provided by the Ruby Menzies and the Australian Postgraduate Scholarships. I would also like to thank the NHMRC and the Cancer Council Tasmania for their grants to implement the mammographic density study, BreastScreen services for their assistance in the retrieval of mammograms, and the Menzies Research Institute, UTAS, for its support and training.

I would also like to acknowledge and thank Fiona Bruinsma, Jo Rayner, Penny Jones, Michelle Kealy, Emma Stubbs, Shirley Catchpole and Tim Albion for their contribution to the data collection (follow-up 1 and 2), questionnaire development, and the administration of the study database.

Thanks also to my friend Julie Cantrill who proof read this thesis. She now knows what I have been doing these past few years.

This study could not have occurred without the study participants. I thank them for their time and effort and hope they find the findings informative.

I would also like to express my thanks to all my friends and family who supported me with encouragement and advice. This includes my colleagues at the Centre for Health Policy, Programs and Economics. And a special thank-you to my Bendigo friends, Jennifer and Chris, who assisted with child pick-ups, drop-offs and school holiday supervision.

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TABLE OF CONTENTS

LIST OF TABLES viii
LIST OF FIGURES xi
PART A 1
1: INTRODUCTION 2
1.1 Research aim and questions 4
1.2 Overview of thesis 5
2: EXPOSURE TO HIGH-DOSE ESTROGENS FOR THE TREATMENT OF TALL STATURE IN ADOLESCENT GIRLS 8
2.0 Introduction 8
2.1 The use of estrogen in the treatment of tall stature 8
2.2 Mechanism of action 13
2.3 Effectiveness of treatment 16
2.4 Other effects of treatment 22
2.5 Overview 36
PART B 39
3: CURRENT EVIDENCE ON THE SHORT- AND LONGER TERM EFFECTS OF ESTROGEN TREATMENT IN ADOLESCENCE ON BREAST SYMPTOMS, DISEASE AND FUNCTION 40
3.0 Introduction 40
3.1 Short-term side effects on the breast 41
3.2 Benign breast disease 47
3.3 Breast cancer risk 55
3.4 Lactation 70
3.5 Overview 80
4: TREATMENT WITH HIGH-DOSE ESTROGENS IN ADOLESCENCE: SHORT-TERM EFFECTS ON THE BREAST AND LONGER TERM BREAST DISEASE 82
4.0 Introduction 82
4.1 Study Aim 83
4.2 Methods 84
4.3 Results 89
4.4 Discussion 97
4.5 Conclusion 103
5: TREATMENT WITH HIGH-DOSE ESTROGENS IN ADOLESCENCE AND SUBSEQUENT EFFECTS ON LACTATION 105
5.0 Introduction 105
5.1 Study Aim 106
5.2 Methods 107
5.3 Results 112
5.4 Discussion 120
5.5 Conclusion 125

PART C 127
6: CURRENT EVIDENCE OF ASSOCIATIONS BETWEEN ESTROGEN EXPOSURES AND MAMMOGRAPHIC DENSITY, A RISK FACTOR OF BREAST CANCER 128
6.0 Introduction 128
6.1 Mammographic density – what is it? 129
6.2 How is mammographic density measured? 129
6.3 Mammographic density and breast cancer risk 134
6.4 Hormone exposures and mammographic density 137
6.5 Adolescent exposures and mammographic density 167
6.6 Overview 170
7: THE LONG-TERM EFFECT OF HIGH-DOSE ESTROGEN EXPOSURE IN ADOLESCENT GIRLS ON MAMMOGRAPHIC DENSITY 175
7.0 Introduction 175
7.1 Study Aim 176
7.2 Methods 177
7.3 Results 197
7.4 Discussion 245
7.5 Conclusion 255
8: CHILDHOOD AND ADOLESCENT GROWTH PARAMETERS, AND MAMMOGRAPHIC DENSITY IN TREATED AND UNTREATED WOMEN 257
8.0 Introduction 257
8.1 Study Aim 260
8.2 Method 261
8.3 Results 265
8.4 Discussion 278
8.5 Conclusion 283
PART D 285
9: CONCLUSION 286
REFERENCES 293

LIST OF TABLES

Table 2.1: Tanner stages of breast maturation. 24
Table 2.2: Summary of mean total plasma IGF-I (ng/ml) before, during and after ethinyl estradiol treatment for tall stature reported in five studies..... 27
Table 2.3: Changes in prolactin concentration (mean ± SD) during ethinyl estradiol treatment for tall stature compared with values before treatment (μg/ml)..... 31
Table 2.4: Summary of studies reporting non-breast related short-term side effects of treatment with high-dose estrogens for in adolescent girls 33
Table 3.1: Breast related side effects of high-dose estrogen treatment for tall stature in adolescent girls..... 43
Table 3.2: Pooled analyses of epidemiological studies examining the association between HRT use and breast cancer risk. 60
Table 3.3: Summary of studies that have investigated the association between environmental estrogen exposures as measured by serum or breast milk concentrations and breastfeeding duration or initiation. 72
Table 3.4: Summary of animal studies on the effect of *in-utero* and prepubertal estrogen exposures on the mammary gland. 77
Table 4.1: Characteristics of treated and untreated participants. 89
Table 4.2: Growth characteristics of treated and untreated participants. 91
Table 4.3: Use of hormones for reproductive conditions in treated and untreated women. 92
Table 4.4: Short-term side effects on the breast by drug type..... 93
Table 4.5: Age adjusted relative-risks of ever having had a breast biopsy, surgery, and breast cancer. 94
Table 4.6: Ever had a mammogram for treated and untreated and Australian population (ABS 2001) by age group. 95
Table 4.7: Reasons for last mammogram in treated and untreated women. 96
Table 5.1: Characteristics of study participants. 113
Table 5.2: Breastfeeding commencement. 114
Table 5.3: Breastfeeding duration for those who initiated breastfeeding 116
Table 5.4: Reasons for stopping breastfeeding. 118
Table 6.1: Cross-sectional studies of the association between HRT and percent mammographic density and dense area measured quantitatively. 141
Table 6.2 Cross-sectional studies of association between HRT and mammographic density measured qualitatively. 142
Table 6.3: Longitudinal studies of the association between HRT and percent mammographic density (PMD) measured quantitatively..... 144
Table 6.4 Longitudinal studies of the association between HRT and percent mammographic density measured qualitatively. 149

Table 6.5: Cross-sectional studies of the association between oral contraceptive pill use and mammographic density. 162
Table 7.1: Age and reproductive characteristics of study participants 197
Table 7.2: Anthropometric characteristics of participants. 199
Table 7.3: Use of hormone or related medications by treatment status. 200
Table 7.4: Smoking, alcohol and socio-demographic characteristics of participants by treatment status. 202
Table 7.5: History of reproductive disease in treated and untreated women. 203
Table 7.6: Treatment characteristics (treated women only). 205
Table 7.7: Mean and median values of mammographic density parameters: dense area (cm^2), percent density (%), total breast area (cm^2) and non-dense area (cm^2). 206
Table 7.8: Univariable analysis of the association between treatment status and the outcome variables: dense area cm^2 (sqrt), percent density (%), total breast area cm^2 (log) and non-dense area cm^2 (log). 209
Table 7.9: Univariable analysis of the association between potential influencing factors and the outcome variables dense area (cm^2) (sqrt), percent density (%), total breast area (cm^2) (log) and non-dense area (cm^2) (log) 211
Table 7.10: Univariable analysis of the association between potential influential variables and the outcome variables dense area (cm^2) (sqrt), percent density (%) (sqrt), total breast area (cm^2) (log) and non-dense area (cm^2) (log) 214
Table 7.11: Regression coefficients of univariable and multivariable analysis of the association between treatment status (treated, untreated) and dense area (cm^2) (square root transformed) 223
Table 7.12: Regression coefficient (multivariable) for treatment effect on dense area (sqrt) with and without digital images and breast cancer cases. 224
Table 7.13: Unadjusted and adjusted regression coefficients of dense area (cm^2) (sqrt) by treatment type: diethylstilbestrol (DES) and ethinyl estradiol (EE) 225
Table 7.14: Number and percentage of women aged before and after 50 years by treatment type. 226
Table 7.15: Regression coefficients for duration of treatment on dense area (cm^2) (sqrt) in treated women after adjustment for age and BMI. 227
Table 7.16: Regression coefficients of unadjusted and adjusted analysis of the association between treatment status (treated, untreated) and percent mammographic density (%) (square root transformed) 230
Table 7.17: Unadjusted and adjusted regression coefficients of percent mammographic density percent (sqrt) by treatment type: diethylstilbestrol (DES) and ethinyl estradiol (EE) 231

Table 7.18: Adjusted regression coefficients of associations between percent density (%) (sqrt) and a) duration of treatment and b) estimated mature height (EMH) minus final height (cm). 232
Table 7.19: Unadjusted and adjusted regression coefficients of the outcome variable percent density (%) (sqrt) and the independent variable: age at start of treatment (years) 233
Table 7.20: Univariable and multivariable regression coefficients of the association between treatment and log non-dense area (cm^2) 235
Table 7.21: Unadjusted and adjusted regression coefficients of non-dense area (cm^2) (log) by treatment type: diethylstilbestrol (DES) and ethinyl estradiol (EE) 236
Table 7.22: Adjusted regression coefficients of associations between non-dense area (cm^2) (log) and a) duration of treatment and b) estimated mature height (EMH) minus final height (cm). 237
Table 7.23: Unadjusted and adjusted regression coefficients for treatment effect on total breast area (cm^2) (log transformed) 239
Table 7.24: Unadjusted and adjusted regression coefficients of total breast area (cm^2) (log) by treatment type: diethylstilbestrol (DES) and ethinyl estradiol (EE) 240
Table 7.25: Adjusted regression coefficients of associations between total breast area (cm^2) (log) and a) duration of treatment and b) estimated mature height (EMH) minus final height (cm). 241
Table 7.26: Multiple linear regression of the association between treatment and each of the mammographic measures adjusted for different sets of covariates. 243
Table 7.27: Adjusted least square means of total breast area (cm^2), non-dense area (cm^2), percent density (%) and dense area (cm^2) for treated and untreated women. 244
Table 8.1: Anthropometric characteristics of treated and untreated participants. 266
Table 8.2: Childhood anthropometric characteristics of treated women. 267
Table 8.3: Univariable analysis of the association between pre-treatment anthropometric measures and the outcome variables dense area (cm^2) (sqrt), percent density (%), total breast area (cm^2) (log), and non-dense area (cm^2) (log). 269

LIST OF FIGURES

Figure 1.1: Study design, outcomes and thesis structure. 7
Figure 2.1: Growth plates at both ends of a long bone 14
Figure 2.2: Tanner stages of human breast maturation. 23
Figure 3.1: Aberration of Normal Development and Involution (ANDI) classification of benign breast diseases. 48
Figure 3.2: Estrogen and breast cancer risk: studies of association presented in the following review 56
Figure 3.3: Pathways for estrogen carcinogenesis. 66
Figure 4.1: Number of treated and untreated women in the Tall Girls Study who were identified, traced and who participated in the computer assisted telephone interview (CATI). 85
Figure 4.2: Proportion of treated and untreated women who had ever had a mammogram 96
Figure 5.1: The breastfeeding questions asked in the computer assisted telephone interview. 108
Figure 5.2: Mean breastfeeding duration (weeks) for treated and untreated women 115
Figure 6.1: Mammograms showing dense (white) and non-dense (dark) areas of the breast across varying degrees of density. 129
Figure 6.2: Representative mammograms of each of the Wolfe Grades of mammographic density measurement. 130
Figure 6.3: Image with the breast area (red) and dense area (green) outlined. 132
Figure 6.4 Study specific and combined relative risks of breast cancer (incidence and prevalence) with increasing percent mammographic density.. 136
Figure 7.1: Flow chart of recruitment of study participants 180
Figure 7.2: Sample size and difference in population means for study powers of 0.8 and 0.9. 181
Figure 7.3: Cranio-caudal and medio-lateral mammogram views. 183
Figure 7.4: Masked (A) and verified (B) images of mammograms. 185
Figure 7.5: Distribution of dense area, percent density, total breast area, non-dense area. 207
Figure 7.6: Distribution of square root transformed dense area and percent density, and log transformed total breast area and non-dense area 208
Figure 7.7: Box-plots of dense area and percent density by menopausal status and age category (<50 years, ≥50years) for treated and untreated combined. 216
Figure 7.8: Total breast area (cm^2) and non-dense area (cm^2) by menopausal status and age category (<50 years, ≥50years) for treated and untreated combined. 217
Figure 7.9: Box-plot of dense area (cm^2) and BMI kg/m^2 for treated and untreated combined. 218
Figure 7.10: Box-plot of percent density (%) and BMI kg/m^2 for treated and untreated combined. 219

Figure 7.11: Box-plot of total breast area (log) and BMI kg/m^2 for treated and untreated combined. 220
Figure 7.12: Box-plot of non-dense area (log) and BMI kg/m2 for treated and untreated women combined. 221
Figure 7.13: Smoothed lowess plot of dense area (cm^2) (sqrt) and age at start of treatment (years) 228
Figure 7.14: Smoothed regression and lowess curves for percent density (%) (sqrt) (y-axis) and the age at beginning of treatment (years) in treated women. 233
Figure 7.15: Smoothed regression and lowess curves for non-dense area (log) (y-axis) and the age at beginning of treatment (years) in treated women. 238
Figure 7.16: Smoothed regression and lowess curves for total breast area (log) (y-axis) and the age at beginning of treatment (years) (x-axis) in treated women. 242
Figure 8.1: Directed acyclic graph illustrating confounding of the association between treatment and mammographic density by pre-exposure variables. 258
Figure 8.2: Directed acyclic graph illustrating a non-confounding scenario of the association between treatment and mammographic density. 258
Figure 8.3: Scatter plot of age at start of treatment and height change after age 15 years. 268

PART A

Chapter 1: Introduction

Chapter 2: Exposure to high-dose estrogens for the treatment of tall stature in adolescent girls

1: INTRODUCTION

Since the 1950s, large doses of estrogen have been used as a treatment to reduce the adult height of tall adolescent girls for psychosocial reasons[5]. Estrogen is believed to contribute to the cessation of bone growth by promoting the ossification of the epiphyseal growth plate of long bones[6] in late puberty[7]. This effect of estrogen is believed to be the basis for its clinical use to treat tall girls[8]. Although the practice is uncommon now, a recent survey of US paediatric endocrinologists reported that 96 (23%) of 411 respondents had treated girls in the preceding five years[3]. In Australia, the treatment regimen typically involved a daily dose of estrogen, either diethylstilbestrol (DES) or ethinyl estradiol (EE), and a progestagen over several days a month to promote cyclical bleeding.

The timing of this treatment in girls has presented a unique opportunity to study the effects on the breast of adolescent exposure to high-dose estrogens. Puberty in girls is an important stage of mammary development and involves the balanced and integrated action of a range of hormones that include the direct and indirect actions of estrogen on primary ductal growth followed by progesterone, alone or together with estrogen, on lobulo-alveolar development[9].

Treatment with high-dose estrogen for tall stature is known to alter the milieu of a range of important hormones. Changes in the levels of IGF-I[10-13], DHEA-S[13, 14], testosterone[13], basal and GnRH stimulated gonadotropins[15, 16], prolactin[13, 17-19] and cortisol[13] have been reported in treated girls. It is possible the hormonal changes observed during treatment for tall stature may affect breast development and subsequently breast histology, function and disease outcomes, particularly since a number of these hormones (e.g. IGF-I) have been implicated in breast development and differentiation[20, 21].

While short-term effects on the breast have been reported in girls receiving estrogen treatment[3, 22-24], no study has quantified these effects by treatment type (DES or EE). Furthermore, no studies have investigated the long-term effects of treatment on the breast. A recent study has shown long-term impaired fertility in treated girls[25], pointing to the possibility of lasting effects on developing reproductive tissues. Animal studies suggest long-

term changes to nipple structure (in primates[26], heifers[27] and goats[28]) and lactation levels (in heifers[27]) when exposed to estrogen during the allometric growth period of the mammary gland that corresponds to the pubertal growth period in girls. It is possible that girls treated for tall stature similarly experience long-term effects on mammary structure and hence function (i.e. lactation).

Increased breast disease risk may be an adverse outcome of treatment. Benign breast lumps have been reported to be a side effect of treatment with high-dose estrogens in adolescent girls[23, 29]. It is possible that these exposures have long-term consequences on breast morphology and subsequent disease.

The effects of hormone exposures during adolescence on breast cancer risk are of particular interest to the scientific community[30]. The adolescent period is considered a potentially important period for breast cancer risk. Evidence supporting a heightened sensitivity during the adolescent period comes from findings in a rat model of carcinogenesis where cancer initiation required the integration of chemical carcinogens with undifferentiated and highly proliferating mammary epithelium[31]. The cells of the pubertal mammary gland are highly proliferative and undifferentiated. Differentiation of the mammary gland, such as that induced by full-term pregnancy, was found to inhibit carcinogenic initiation.

Mammary cellular proliferation is stimulated by exposure to estrogen and progesterone[32, 33]. It has been suggested that lower levels of these sex hormones during adolescence could potentially protect against breast cancer by altering breast morphology through a reduction in the rate of cell turnover and proliferation[34]. Early age of menarche is a well established breast cancer risk factor[35] and this may be attributable to longer or earlier lifetime exposure to estrogen and progesterone, especially during the critical period of breast development.

While adult exposures to exogenous sex hormones and their influence on breast cancer risk have been studied extensively, no studies have examined the effect of adolescent exposure to supraphysiological doses of estrogen on the risk of breast cancer in women. A cohort study of Australian tall girls presented an opportunity to investigate whether

adolescent exposure to high-dose estrogens influences breast cancer risk by comparing the number of breast cancer cases and mammographic density in treated and untreated women. The sample size of this cohort was a limitation but it was worth examining cases of breast cancer to rule out a large increase in risk in these women. Mammographic density is a well established determinant of breast cancer[4] and can be considered a proxy measure of breast cancer risk[36]. The sample size of the cohort was large enough to explore the difference in mammographic density between treated and untreated women.

Treatment with sex hormones in adult women (hormone replacement therapy, Tamoxifen, and Gonadotropin Releasing Hormone Agonist (GnRHA), has been demonstrated to influence mammographic density[37-39]. However, the effect of these treatments may be only temporary[39, 40]. The effect of sex hormone treatments during adolescence is not known but it is plausible that exposures during pubertal mammary gland development have a more sustained influence on breast tissue composition and mammographic density.

The Tall Girls Study cohort provided a unique opportunity internationally to examine the effects of adolescent exposure to high-dose estrogens on the breast. This cohort comprised Australian women who were assessed for tall stature as adolescents, had a wrist x-ray to predict estimated mature height, and were either treated or untreated.

1.1 Research aim and questions

The aim of this research was to examine the short and long-term effects of adolescent exposure to high-dose estrogens on the breast, in particular: the short-term side effects of treatment on the breast, and longer term effects on breast disease, mammary function (lactation), and mammographic density. To achieve this aim, the following research questions were developed:

1) What proportion of the women in the Australian Tall Girls cohort experienced breast related short-term side effects? What were they and did they differ by treatment type?

2) Were women treated for tall stature more likely to develop breast cancer than women assessed for tall stature but not treated?

3) Were women treated for tall stature more likely to have had a breast biopsy or undergone breast surgery than women assessed for tall stature but not treated?

4) Were women treated for tall stature more likely to not commence breastfeeding; to breastfeed over a shorter duration, both in total and exclusively; or to differ in their reasons for stopping breastfeeding when compared with women assessed for tall stature but not treated?

5) Were women treated for tall stature more likely to have a higher breast density for their age, than women assessed for tall stature but not treated?

To answer the first four or these questions, data that was previously collected in the Tall Girls Study (follow-up 1) were analysed. To answer Question 5, eligible women from follow-up 1 were retraced and recruited to a second follow-up (follow-up 2) and new data were collected from these women.

As well as helping to address the concerns of treated women, the research provided a rare opportunity, internationally, to examine the long-term biological effects of this treatment. While exposure to supraphysiological doses of estrogen in adolescence is not common now, it is important to establish whether exposure to high-doses of sex hormones during adolescence is likely to have a sustained effect on the breast.

1.2 Overview of thesis

This thesis is composed of five parts. Part A includes Chapters 1 (this chapter) and 2. The second chapter provides the background to the treatment of tall girls with high-dose estrogens. Specifically, it examines the literature on the treatment of tall girls, its indication of use, current use, treatment regimen, mechanism of action and effectiveness. The effect of treatment on pubertal characteristics is described, as is the effect of treatment on hormone levels (e.g. estradiol, growth factors, DHEAs and gonadotropins and prolactin).

Part B describes the research that involved an analysis of the data collected in follow-up 1 and addresses the research Questions 1–4 above. This part includes Chapters 3, 4 and 5 **(See Figure 1.1).**

Chapter 3 reviews Australian and international published case-series and follow-up reports of adverse effects on the breast during and shortly following cessation of estrogen treatment in tall girls. It explores available evidence, drawn from animal and epidemiological studies, of the effect of hormones during all stages of mammary development, particularly the adolescent period, on breast morphology, function and disease outcomes. The review highlights the gaps in our understanding of the short- and long-term effects of high-dose estrogen treatment in adolescence on breast symptoms, disease and function.

Chapter 4 describes the prevalence of short-term breast related side effects in treated women, and the long-term risk of ever having had a breast biopsy, breast surgery, and breast cancer in treated women compared with untreated women at first follow-up.

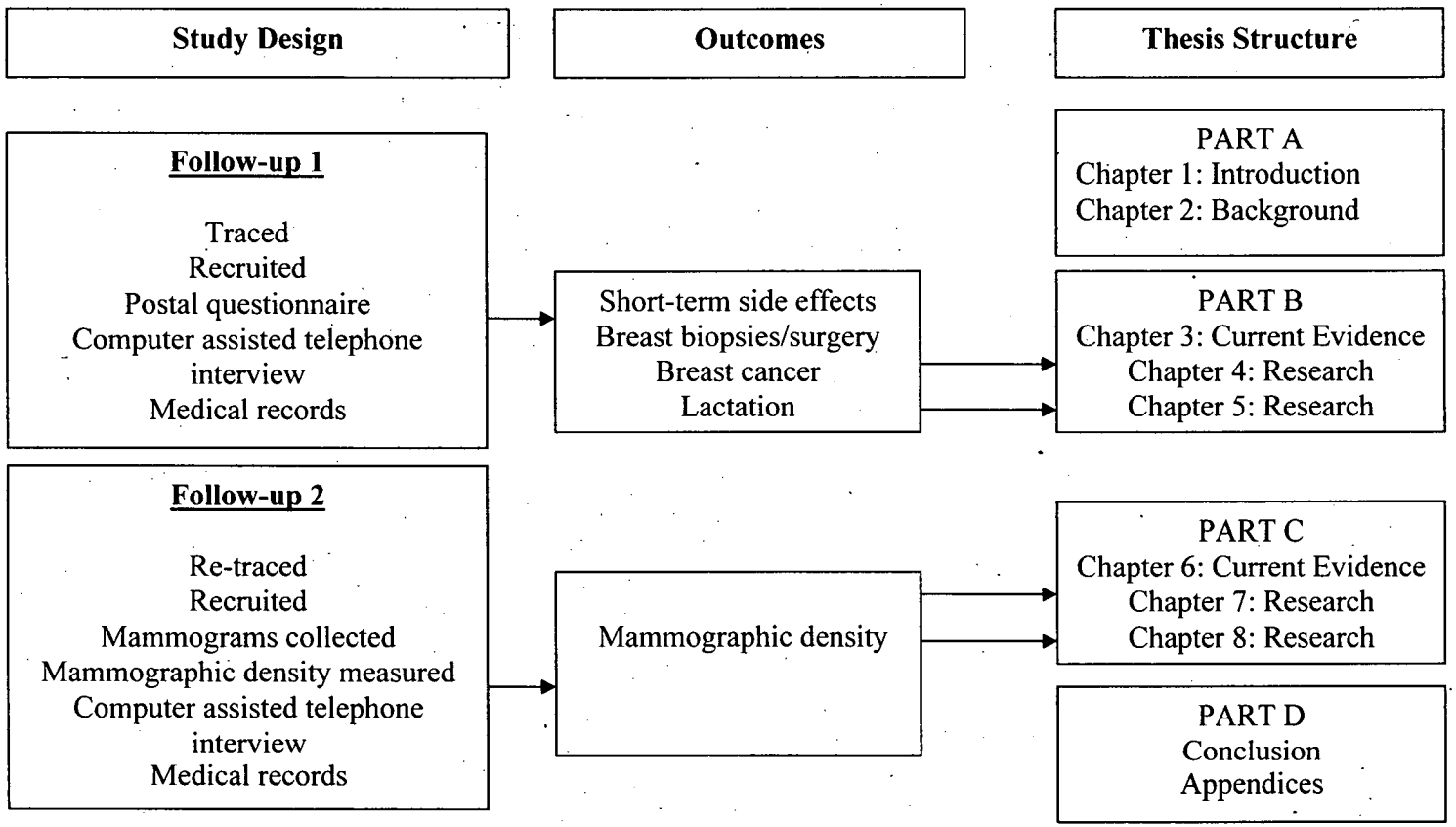
Chapter 5, the final chapter in Part 2, describes the long-term effects of high-dose estrogen for the treatment of tall stature in adolescent girls on subsequent lactation, in particular, breast feeding commencement and duration at first follow-up.

Part C describes the research involving follow-up 2, and includes Chapters 6, 7 and 8. Chapter 6 presents a review of the literature on mammographic density, an important breast cancer risk factor, and evidence of hormone exposures and their effects on mammographic density.

Chapter 7 follows with the research findings of the study on the effects of high-dose estrogen treatment in adolescent girls on mammographic density at second follow-up. Chapter 8 continues with the examination of the influence of pre-treatment and post-treatment anthropometric parameters on the association between treatment and mammographic density.

The overall findings are evaluated and discussed and conclusions drawn in Chapter 9 (Part D), followed by the Appendices.

Figure 1.1: Study design, outcomes and thesis structure.



2: EXPOSURE TO HIGH-DOSE ESTROGENS FOR THE TREATMENT OF TALL STATURE IN ADOLESCENT GIRLS

2.0 Introduction

This chapter provides the background and context to the research presented in this PhD thesis. Specifically, this chapter reviews the use of estrogens in the treatment of tall stature in adolescent girls: its indications for use, current use, treatment regimen, mechanism of action, and effectiveness.

This chapter then follows with an exploration of the effect of estrogen treatment for tall stature on pubertal characteristics and endogenous hormone levels using evidence sourced from the literature. Reports of short- and long-term side effects of treatment are also described.

The reported effects of treatment on pubertal characteristics, hormonal levels and reproductive organs, together support the suggestion that treatment with high-dose estrogens in adolescence could have long-term effects on the breast.

2.1 The use of estrogen in the treatment of tall stature

Estrogens have been used to reduce the adult height of tall girls since the 1950s. The first clinical use of estrogen treatment in tall but otherwise healthy girls appeared to be explored in 1946 at the Massachusetts General Hospital, as an extension of the treatment of acromegaly[41]. Interest in the treatment increased one decade later (1956), when US based Goldheizher published the first clinical observations of a cohort of treated female patients[5]. At this time, Australian endocrinologist Norman Wettenhall, whilst visiting the US, became interested in the use of high-dose estrogens in the treatment of tall girls, and became a highly published proponent of the treatment in Australia and internationally.

The use of estrogen in the treatment of tall stature has been the subject of many published articles on its clinical use in limiting the height in girls[42-46] and, more recently, its safety in relation to possible long-term side effects[42, 47, 48] and the ethical issues concerning treatment[49]. A lack of knowledge about the long-term effects has led to recommendations in the clinical, scientific and wider communities (e.g. Tall Girls Inc.*) for the implementation of long-term follow-up studies[23, 44, 47, 50]. In response to this need, a group of Australian researchers, based in Melbourne, recruited an Australian cohort of treated and untreated women who were assessed for tall stature in adolescence to examine the long-term effects of treatment, the results of which will be described in a later section of this chapter.

2.1.1 Indication for use of high-dose estrogens in adolescent girls

The indications for use of treatment have varied over time. Girls with an expected mature height exceeding between 177–188 cm[3, 51], depending on the decade of assessment, or >2SD for age [44, 52], and presenting with concerns about adverse psychosocial effects of tall stature, have been offered estrogen treatment for psychosocial benefits[3, 51, 52] if at an age when treatment might still be effective. In 1965, Wettenhall and Roche, described some of the psychosocial problems faced by tall girls and said to justify treatment[52]:

"A kyphotic posture may be adopted defensively to reduce their tall appearance; depression, withdrawal from social contacts, lack of interest in school work and play, [and] tensions and irritability at home, are common emotional reactions."

"Some girls feel so embarrassed with boys shorter than themselves that they believe their choice of male companions, both in the immediate future and as adults, will be seriously jeopardised."

"They may have difficulty in buying clothes appropriate to their age, and if clothes have to be tailor-made, extra expenses can be a problem."

"Some careers, for example classical ballet, are closed to an unusually tall girl." p 210.

* Tall Girls Incorporated is an Australian advocacy group for women who were treated with estrogens to reduce their final height in adolescence.

While the psychological benefits of treatment to the child are one of the criteria for treatment, the parents' own anxieties have been reported to influence the decision for treatment as described by Zackman[46]:

> *"The parents who usually are also excessively tall may be alarmed because they remember their own psychological sufferings as adolescents and young adults and fear that their child may have difficulties in finding a partner. In such situations, the possibility of an effective treatment is enthusiastically accepted, even if the effect is small and even if there is a theoretical risk."* pp 13–14.

Or as Wettenhall stated[53]:

> *"The concern may originate with the parents and the child may not be bothered at all."* p 135.

Of 844 Australian women who were treated with high-dose estrogens for tall stature, only 17.5% reported "their own unhappiness or difficulties" as the reason for seeking a medical assessment of their height[54]. For most of the girls, according to Bruinsma et al. (2006)[54], it was a parent and/or doctor who found the predicted final height to be an issue.

2.1.2 Prevalence of use

Although currently available, estrogen as a treatment for tall stature in girls is not as popular as it once was. This is likely to be due to the growing acceptability of tall stature in females[3, 41, 42], and the waning acceptability of hormone interventions to reduce tall stature fuelled by the concern about potential side effects[3]. The extent of its use in the treatment of tall stature in Australia is not clear, as there are no studies reporting the prevalence of treatment, however, a recent study of US paediatric endocrinologists found that 33% of respondents still offered hormone treatment for tall stature in girls while 22% had treated girls for tall stature during the preceding five years[3]. Subsequent

findings from the Australian Tall Girls Study suggesting long-term adverse effects on fertility, may have led to this treatment becoming even less popular[25].

The treatment is currently offered in Europe. An article published in The Age newspaper in 2007[55] described the increasing trend in population height in the Netherlands and included an interview with a paediatric endocrinologist from Groningen, northern Holland. In the interview, the endocrinologist stated that he treated about one child per week with hormones for tall stature (boys† and girls) despite the warnings about the greater risk of long-term fertility problems in treated girls:

> *" There are no medical reasons for intervening in [the growth of] these kids...The reasons are practical and cosmetic...If a girl is estimated to grow over 185 cm..., then we offer them the choice [of treatment]... We don't know the long-term side effects...All we have is an Australian study which found treated women were more likely to have fertility problems. I always make sure I mention that."*

While use of estrogen for tall stature may have waned over the years, a study published in 2008[56] that had examined the haemostatic effects and clinical effectiveness of treatment, promoted the continued use of high-dose estrogens in the treatment of tall stature in girls.

In addition to its use in constitutionally tall girls, the use of high-dose estrogens to reduce the height of children with profound developmental disabilities as a way of facilitating their management; has recently been suggested[57] and debated[49].

2.1.3 Treatment regimen

The type of estrogen used has varied over time and place. In the US, conjugated equine estrogens are the current estrogens of choice, while in Europe and Australia it is ethinyl

† While rare, boys are generally treated with testosterone (T) ester depot preparations (250–1000 mg/month for tall stature (Drop et al. 2001)[50].

estradiol. Diethylstilbestrol was previously used until it was reported in 1971[58] to be associated with rare clear cell adenocarcinoma of the vagina in the daughters of women treated with diethylstilbestrol in pregnancy to prevent miscarriage. As part of the regimen, a progestagen is typically prescribed to induce cyclic bleeding and avoid over stimulation of the endometrium and has included norethisterone, medroxyprogesterone and dydrogesterone[44].

Doses have varied over time. According to Drop et al. (1998)[44] ethinyl estradiol was used in doses of 500 μg in the 1960s reducing to 200–300 μg in the 1970s, with some using a dose of 100 μg in the 1990s. Some studies have examined the use of lower doses[59] but these have not yet come into practice.

In Australia, from 1959 to 1971, girls were typically treated with diethylstilbestrol (DES) (typically 3 mg/day), combined with a progestagen (typically norethisterone, 5 mg twice a day for 4 days per month). After this time, DES was replaced with ethinyl estradiol (EE) (typically 150 μg/day). Wettenhall recommended the use of ethinyl estradiol after the discontinuation of DES in 1971, because the alternative, Premarin (conjugated equine estrogens), used in the US, had varying amounts of estrogen in different production batches[60]. He recommended the dose 150 μg/day because it equated with the DES dose in terms of potency. According to Wettenhall, ethinyl estradiol has a potency approximately 25 times greater than DES. This equated to a dose of 120 μg/day EE, but was increased to 150 μg because the tablets were in doses of 50 μg[60].

Ethinyl estradiol is the major component of the contraceptive pill, and still the estrogen of choice in the treatment of tall stature in girls. As an indication of the size of the dose, the treatment dose of ethinyl estradiol for tall stature in adolescent girls is approximately five times the dose used in the oral contraceptive pill (contains between 20–30 μg/day[61, 62]). Clearly supraphysiological doses of estrogen were used in the treatment of tall stature.

2.2 Mechanism of action

The mechanism of action of high-dose estrogen on growth in adolescent girls is unclear though evidence suggests it has a direct or IGF-I mediated effect on the growth plate of long-bones as described below.

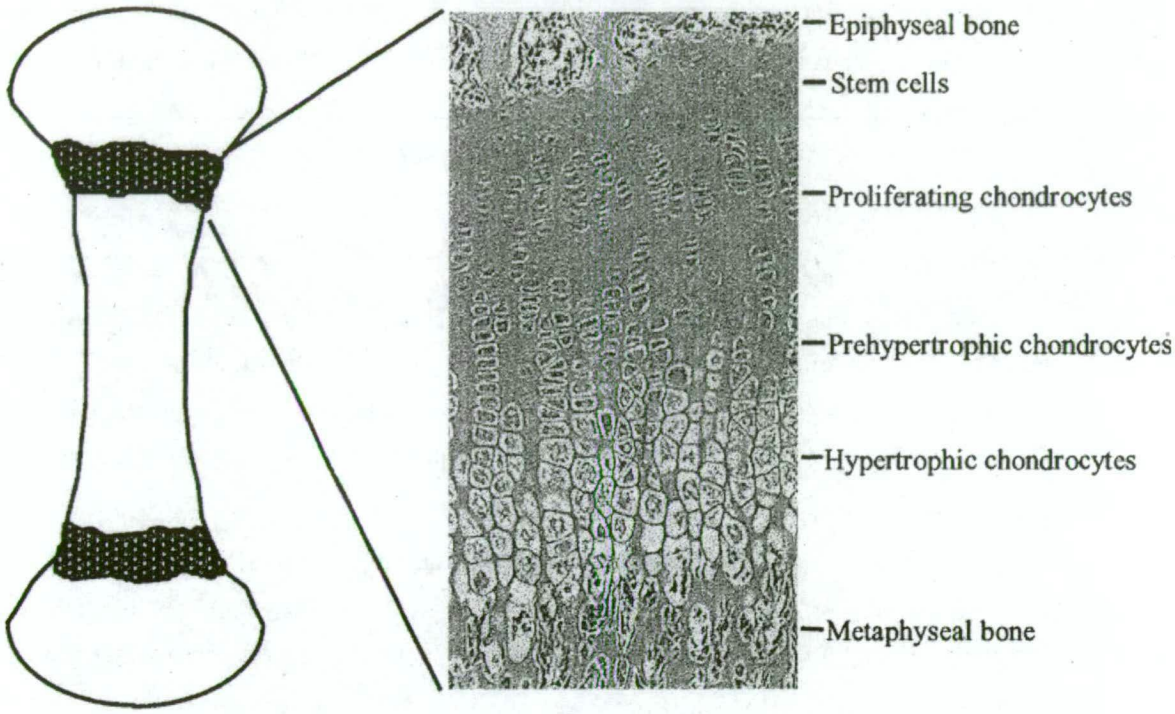
2.2.1 Biphasic-effect

Estrogen has a number of roles during puberty, one of which is to stimulate the maturation of the long-bones in late puberty, which leads to a cessation in growth[7]. This is in contrast to the effects of estrogen during the early pubertal growth spurt, where it is known to stimulate growth[7, 63]. The dual role is believed to depend on the level of endogenous estrogen, with lower levels stimulating growth and higher levels preventing further growth[7]. This is supported by *in vitro* studies showing a biphasic action of estrogen on the proliferation of human chondrocytes. At low concentrations, estrogen appeared to stimulate proliferation of chondrocytes while at supraphysiological doses, proliferation was inhibited[64].

2.2.2 Growth plate as the target tissue of estrogen

Estrogen is believed to contribute to the cessation of bone growth by promoting the ossification of the epiphyseal growth plate of long bones[6] in late puberty[7]. This effect of estrogen is believed to be the basis for its clinical use to treat tall girls[8] (See **Figure 2.1** for a diagram of the growth plate and proliferating chondrocytes).

Figure 2.1: Growth plates at both ends of a long bone.



Source: Van Der Eerden B.C.J, & Wit, J.M (2003)[65] Permission to reproduce granted.

The use of estrogen to promote epiphyseal fusion in tall girls is also founded on the observation that short-stature is common in children with precocious puberty. Girls with precocious puberty have an early onset of puberty (before 8 years of age)[66]. These children are tall at an early age due to the earlier pubertal growth spurt, but become short adults due to premature epiphyseal fusion[66].

Further support of a direct action of estrogen on the growth plate is the presence of estrogen receptor-alpha (ER-α) and estrogen receptor-beta (ER-β) within the growth plate and adjacent bony tissue of children in the prepubertal and pubertal age period[67]. Weise and colleagues proposed that estrogen acts by reducing the proliferative potential of the growth plate through a process of chondrocyte exhaustion[68]. It has been suggested

elsewhere that estrogen might accelerate chondrocyte senescence by a proliferation-independent mechanism[69].

2.2.3 IGF-I as a mediator

While the mechanism of action of high-dose estrogen on growth in adolescent girls is yet to be made clear, growth hormone or growth factors may have a role to play[12, 44]. A review of the literature suggests insulin-like growth factor (IGF-I) (previously known as somatamedin-C) is particularly important to pubertal growth[70] and is mediated by estrogen[71]. In children and adolescents, low doses of estrogen (up to 0.030 mg) have been found to stimulate serum insulin-like growth factor-I (IGF-I), while higher doses (greater than 0.100 mg) have been found to suppress IGF-I[10]. This parallels the pattern of growth seen with low- and high-dose estrogens as described earlier.

One group of investigators[11] disagreed with the IGF-I mediating role on the growth plate, and suggested that other factors were at play. This conclusion was drawn after studying the effect of three doses of estrogen on both height reduction and IGF-I levels. While height reductions were observed for all doses (0.25, 0.5 and 1.0 mg), a significant reduction in IGF-I was only observed for doses 1.0 mg and 0.50 mg. A reduction was observed for a dose of 0.25 mg but this was not statistically significant. No confidence limits were provided to gauge the precision of this result. This finding contradicts other studies[10, 12, 13], however, and may in part be due to a lack of power in the sub-sample analysis, or the cyclic rather than continuous nature of the estrogen used. Three week cycles, rather than continuous daily doses, were used in the former study. It is also not clear whether the timing of the IGF-I measurements was consistent across the different doses of estrogen used in the study.

Zackman et al. (1975)[46] argued against the IGF-I mediating view on the basis that patients with IGF-I deficiency have been shown to have retarded bone maturation. Zackman presented an alternative mechanism, suggesting that epiphyseal plate maturation is a result of androgenic action stimulated by the high-dose estrogen. No subsequent articles on this topic have re-visited this potential mechanism.

Cortisol levels may have a role to play. As described later in this chapter, treatment with high-dose estrogens has been observed to increase the level of circulating cortisol in girls, and that, according to Minuto et al. (1989)[70], cortisol exerts a direct inhibitory effect on cartilage replication as seen in subjects with Cushing syndrome or receiving corticosteroidal treatment. Despite this observation, no known reports on treatment of tall girls have mentioned this as a potential mechanism of action.

The growth inhibiting effect of estrogen appears to be mediated through its action on the growth plate, however, it is unclear whether this is a direct effect or mediated through its inhibitory action on IGF-I levels. The actual mechanism of action on the growth plate is still to be determined.

2.3 Effectiveness of treatment

The following section explores the effectiveness of treatment in achieving both a reduction in final height and improved psychosocial outcomes.

2.3.1 Effectiveness on final height reduction

The primary intended clinical outcome of high-dose estrogen treatment in adolescent girls is a reduction in the girl's final height. Effectiveness of treatment in reducing final height is measured by calculating the difference between the estimated mature height (EMH) and final height. This section describes the measurement of treatment effectiveness, using EMH, and summarises the findings of studies that have examined the effectiveness of treatment using this measure.

2.3.1.1 Measuring final height reduction

Estimated mature height is a prediction of the final height calculated prior to treatment, and is used to determine the remaining growth potential of the girl, and therefore the potential for treatment. EMH can be measured by calculating bone age, and extrapolating EMH from this measure, using standardised tables of predicted heights, based on growth

data of children (e.g. Bayley-Pinneau tables[72] or Tanner-Whitehouse tables[73]). Drop et al. (1998)[44] summarised the techniques of height prediction using bone age in their review of the management of tall stature. The following discussion on the bone age estimation is drawn from this review with additional sources from the research literature.

The tables for extrapolating final height use different methods of bone age estimation. Bone age is calculated by evaluating radiographic images of the wrist using one of a number of methods[73-75] of varying accuracy[75]. One method uses the Greulich and Pyle atlas to estimate bone age. This atlas provides standard radiographs of the left hand and wrist for different ages. The bone age of the child is determined by comparing their radiograph with the standard. The Bayley-Pinneau tables for predicting final height (EMH) uses this method of bone age assessment. Another form of bone age assessment is the Tanner-Whitehouse technique. This technique also uses a number of maturity indicators, weighted and scored, for each bone of the hand and wrist. These individual scores are added to form an overall bone age score. The children selected for these standards were from average socio-economic strata and the UK, whereas those selected for the Greulich Pyle were from high socioeconomic strata in the US and white. The Tanner-Whitehouse height (EMH) prediction tables referred to above use this method of bone age assessment. Bone age estimated using the Greulich and Pyle method has been reported to be approximately one year lower than the bone age estimated by the Tanner-Whitehouse method[44].

According to Drop et al.(1998)[44] both techniques of bone age assessment are subjective, and this subjectivity is reflected in the inter- and intra-rater variability. The Bayley and Pinneau tables were used to estimate mature height in the Australian patients of Wettenhall. This method utilises the Greulich-Pyle atlas for bone age assessment. Between 1959 and 1975, for all patients, the bone age of the hand-wrist was assessed by one observer. The mean intra-observer difference in assessments was calculated to be 1.1 months[60].

If EMH and final height data are known, treatment effectiveness can be calculated. Treatment effectiveness is determined by calculating the difference between EMH and final height. In treated girls, a final height that is lower than the EMH, exemplifies effective treatment. A final height that is equal in magnitude, or higher than the EMH, suggests the treatment was ineffective.

2.3.1.2 Reports of effectiveness of treatment in reducing final height

Published estimates of the effectiveness of high-dose estrogen treatment in reducing adult height vary widely[44]. According to Drop et al. (1998)[44], this high variability is due to differences in the height prediction methods used, treatment regimens, and timing of treatment between studies[44].

In a review of 17 studies, Drop and colleagues reported corrected estimates of height reduction ranging from 2.1 to 10 cm[44]. Corrections were based on systematic prediction errors, as reported in the literature. Since this time at least five studies on the effectiveness of treatment have been reported[22, 48, 56, 76, 77] with mean height reductions of between 2.4–5.5 cm, though only one of these studies, Venn et al. (2008)[77], adjusted for error in EMH predictions. Venn and colleagues examined treatment effect (final height *minus* EMH) in the Australian cohort of treated girls, by adjusting for error in EMH predictions and the different distributions, using pairs of treated and untreated girls matched on their EMH within 1 cm[77]. In the treated group, the mean difference between final height and EMH was found to be −1.4 cm (SE 0.29) while the difference was +1.1 cm (SE 0.23) in the untreated group, equating to an unadjusted treatment effect of −2.5 cm (95% CI: −3.2 to 1.8) (n=279)[77].

2.3.1.3 Effectiveness in relation to timing of treatment

The significance of timing of treatment has been raised with observations by some, that treatment is more effective if started before menarche[78], at an earlier bone age [44, 50, 56, 77] or chronological age[22, 50, 56]. In relation to menarche, Kuhn et al. (1977)[43] did not find any difference in results when therapy was started before or after menarche. This is supported

by Drop et al. (1998)[44]. While premanarcheal girls seemed to benefit more from therapy than postmenarcheal girls, on further adjustment, they found this difference to be due to chronological age rather than timing of treatment in relation to menarche.

While reports of treatment being more effective the younger the child at start of treatment, Wettenhall argued that treatment before 10 years should be avoided because treatment induced menstruation is undesirable in girls under this age[60].

Some studies[13, 41] have reported delaying treatment until girls were at least as far advanced in puberty as Tanner Stage 3 (see below for a description of Tanner stages of the breast). No studies appear to have reported effectiveness in relation to Tanner stage at start of treatment.

2.3.2 Post-treatment growth

Wettenhall, in the textbook *Clinical Paediatric Endocrinology* (1981)[53] described the criteria for stopping treatment as follows:

> *"A girl's progress is reviewed monthly for the first three months [following treatment] and thereafter three or four times a year.... When her height has remained unchanged for six months, radiology is done to determine the state of epiphyseal maturation, treatment being continued until epiphyseal fusion is complete."*

Growth is not necessarily complete following treatment. Additional growth of a mean 2.7 cm on cessation of treatment has been observed[79]. There are two possible reasons for this additional growth[44]. Firstly, cessation of therapy may have been premature before complete closure of the epiphyses. Secondly, the additional growth may be due to vertebral growth as this growth is not affected to the same degree by estrogen's action on long bone growth[6, 80].

2.3.3 Psychosocial outcomes

While psychosocial factors are often cited as the main reason for treating tall stature, there is no clear evidence supporting lifelong psychological damage from being tall[44, 50, 81, 82], though short-term problems may be experienced in adolescence. Lecointre and Toublanc (1997)[82] examined the benefits and disadvantages of being tall by surveying 113 French women whose height was between 175–188 cm (>2 SD of the population). As an adult most of the tall women studied were satisfied with their height 85% (175–179 cm), though fewer were satisfied with their current height if they were 180–188 cm (69%)[82]. The women stated the advantages and disadvantages of being a tall woman. According to the investigators:

> "*In the great majority of cases, tall women describe difficulties mainly with relationships during adolescence and emphasize the crucial contribution of family members in providing psychological support. These difficulties may disappear in adulthood when the woman has overcome the disadvantages felt in adolescence and has succeeded in taking advantage of her tall stature in her professional life. Tall stature is striking and if a woman can respond appropriately, she may gain autonomy and sometimes advantage by her height.*"[82] p 531.

While the above study identified relationship difficulties, particularly in adolescence, one study did not find this to be the case. Sandberg et al. (2004)[83] found no relationship between tall height (≥1.6 SD; n=58 of which 25 were girls) and measures of friendship, popularity and reputation with peers in a cross-sectional study of 956 US students. The investigators found that height influenced the peer relationships of boys and girls in similar ways.

A large study[81] surveyed visitors to a news website and Elle magazine (30,347 women, mean age 34 years) using an on-line "Sex and Body Image Survey". They found 80% of women between heights 5'7" and 5'11; 77% of women at 6'; and 60% of women between 6'1" to 6'3" expressed contentment with their heights. Results were similar

across age groups. According to the authors, the low prevalence of dissatisfaction observed among the tall women in their study does not support the use of estrogens to reduce height for psychosocial reasons.

These observations raise the question about the effect of treatment on the longer term psychosocial wellbeing of tall girls. Wettenhall observed psychological improvement in almost all girls treated, because:

> *"they were receiving help in coping with their problems. No doubt the regular discussions with a friendly and interested doctor were appreciated by many of these girls and their parents but, without estrogen therapy as well, these discussions would not have been enough to account for the improvement of their morale."* p 607, paragraph 9.

Only two studies appeared to formally investigate the psychosocial outcomes of treatment. A retrospective study of 56 treated and 79 untreated (control) tall girls by Binder et al. (1997)[84] at a mean age of 21.8 years at follow-up, revealed no major psychosocial or social maladjustment differences between the two groups despite treated women reporting teasing because of tallness more frequently than controls[84].

The Australian Tall Girls Study[54], a retrospective cohort study of 396 treated and 448 untreated Australian women who were assessed for tall stature in adolescence, also examined the long-term psychosocial outcomes of both groups of women (mean age at follow-up 39.1 years). The study found no significant difference between treated and untreated women for a number of psychological outcomes that included 12-month or lifetime major depression, eating disorders, scores on the SF-36 mental health summary scale, or an index of social support. However, compared with population based data, the prevalence of major depression in both groups was high. Self-reported difficulties during adolescence that led to the seeking of a medical height assessment (OR 2.25, 95% CI: 1.4 to 3.6), and a negative experience of the assessment or treatment procedures (OR 2.04, 95% CI: 1.4 to 3.6) were found to be significantly associated with lifetime major

depression. The study investigators concluded that the intended psychosocial benefit of treatment may not have been realised.

Research on women's satisfaction with their treatment can also inform the debate about the benefits of estrogen therapy for tall stature. Weiman and colleagues (1998)[22] retrospectively surveyed 50 treated girls, by questionnaire, for their views of treatment. While 84.6% of the patients were satisfied with treatment, 15.4% regretted having had it. This is despite the unpleasantness of the side effects of treatment: 38.4% recalled the side effects of treatment as unpleasant while 61.5% did not[22]. Binder et al. (1997)[85] also asked 56 treated women about their satisfaction with treatment and found the decision to opt for treatment was retrospectively approved by 95.8%. These two studies, however, had small and selected samples of women who were followed up by the treating physicians as opposed to independent researchers.

The larger Australian Tall Girls Study revealed a higher rate of dissatisfaction with treatment[86]. The study found that 42.1% of treated women expressed dissatisfaction with the decision that was made to treat them while untreated women were almost unanimously glad they were not treated (99.1%), no matter how tall they became. There was no clear association between satisfaction with treatment and women's final height. These findings do not support the rationale for treating tall stature with high-dose estrogens for psychosocial reasons, and highlight the continuing debate about its use.

2.4 Other effects of treatment

As well as height and psychosocial outcomes described above, treatment with high-dose estrogens for tall stature in adolescent girls has been reported to have a number of additional physiological effects. The onset of pubertal characteristics is accelerated, and levels of circulating hormones are altered. Unwanted side effects associated with these changes have also been reported and are described more fully below.

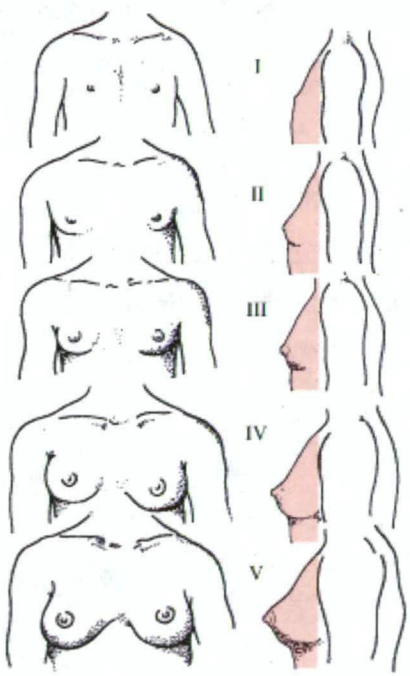
2.4.1 Effect of treatment on pubertal characteristics

Treatment accelerates the onset of secondary sexual characteristics, and onset of menses[41], and it is known to suppress gonadotropin secretion due to a reversible negative feedback mechanism[87]. These and other effects on pubertal characteristics, including the Tanner stages of breast development are described below.

2.4.1.1 Effect on breast development

Pubertal breast development is divided into five stages according to Tanner[88] (see **Figure 2.2** and **Table 2.1**).

Figure 2.2: Tanner stages of human breast maturation.



Source: Marshall and Tanner (1969)[1].

Table 2.1: Tanner stages of breast maturation[88].

Tanner Stage	Breast Characteristics
1	Pre-pubertal
2	Elevation of breast bud Areola diameter enlarged
3	Enlargement of the breast and areola No separation of the breast/areola contours
4	Further enlargement of the breast and areola. Areola forms a secondary mound above the level of the breast
5	Projection of the breast only to final adult size Areola within contour of the breast.

No studies appear to report the effects of high-dose estrogen treatment on the breast in relation to Tanner stages, however, it has been suggested that breast development, along with other pubertal characteristics, is accelerated with treatment[46]. In contrast, two reports suggest the opposite; that treatment impedes breast development[23, 41]. However, these potential effects were self-reported by the treated girls and not based on a clinical assessment (e.g. Tanner stage).

As stated above (Section 2.3.1.3), a series of studies (in Boston, US) described by Crawford[41] started treatment when the tall girls were at least as far advanced in puberty as Tanner's Stage 3. It is unclear whether commencement of treatment in relation to Tanner stage is a determining factor in its effect on breast development.

2.4.1.2 Effect on menses and ovulation

According to Wettenhall (1981):

> *"A girl who has not reached her menarche can expect to do so within three months of the onset of therapy, and those already menstruating develop frequent and often prolonged menses. These disabilities are corrected by adding norethisterone for four days at monthly intervals..."*[53] p 137.

The age at menarche in treated girls who had not yet started menstruation, is artificially induced by treatment[53]. Whether this menstruation is ovulatory or not is unclear. Menstruation without ovulation is described as anovulatory. Normally, there is a period of anovulation months and sometimes years following natural menarche[89]. When ovulatory cycles occur the corpus luteum matures and progesterone is then secreted[89]. While menses is initiated with treatment, ovulation is unlikely during these periods given the suppression of the hypothalamo-pituitary-gonadal axis[87] (described in more detail below). No reported studies have examined the direct effect of high-dose estrogen treatment on ovulation in girls. Animal studies using heifers, suggest that early and continuous treatment with estradiol implants can retard reproductive function as observed by the lack of a palpable corpus luteum or a threshold serum progesterone level[90].

In their review of case-series reports, Drop et al. (1998)[44] reported spontaneous bleeding to occur one to six months following the discontinuation of treatment in patients within these studies[44]. A follow-up study (mean 10 years) by De Waal et al. (1995)[29] of 180 women who were treated in adolescence with high-dose estrogens found 5% had not started menses six months following treatment cessation and 2% had not started menses after 12 months.

2.4.2 Effects of treatment on circulating hormone levels

As well as the expected increase in plasma estrogen levels, a review of the literature has revealed a range of treatment modulating effects on a large number of endogenous hormones. Treatment has been reported to suppress insulin-like growth factor-I (IGF-I),[10-13, 91], dehydroepiandrosterone sulphate (DHEA-S)[13], testosterone[13] and basal and GnRH stimulated gonadotropins[16]; and increase prolactin[13, 18, 19], IGF-II[12] and cortisol[13, 14] levels. Treatment has also been shown to reduce insulin-like growth factor binding protein-2 (IGFBP-2)[10, 12] and increase insulin-like growth factor binding protein-4 (IGFBP-4)[10, 12].

It is necessary to understand the extent to which treatment has affected endogenous hormone concentrations, as these changes may have implications for both short- and long-term effects on the breast, particularly if the hormones have a role in mammary gland development or function. A review of the literature[‡] in relation to these hormonal changes is presented below.

2.4.2.1 Estradiol

Ethinyl estradiol (EE2) was the estrogen used in the treatment of girls for tall stature after 1971. It is stronger in estrogenic potency than estradiol (E2)[16, 92], which is the endogenous form of estrogen. One study measured circulating exogenous ethinyl estradiol (EE2) and endogenous estradiol (E2) concentrations in the blood of seven girls treated with 500 μg/d of ethinyl estradiol[16]. Ethinyl estradiol levels were increased to 470–1100 pg/ml, as expected, but E2 levels were reduced to 17±1.6 pg/ml from a baseline of 36±2.0 pg/ml.

2.4.2.2 Insulin-like growth factor (IGF-I)

Insulin-like growth factor-I (IGF-I) is a polypeptide, similar in structure to insulin. Its release is stimulated by growth hormone (GH). Five longitudinal studies demonstrated a reduction in IGF-I levels with ethinyl estradiol treatment in tall girls (**Table 2.2**). Reductions of between 20%[13] and 34%[12] from baseline were observed within the first six months of therapy with 0.1 mg/day of ethinyl estradiol. A greater reduction was observed with higher doses (e.g. 56% with 1.0 mg/day[11]). Another study[93] (not in table) reported average serum IGF-I (somatamedin) levels falling 56.9% of baseline after six months of treatment with conjugated estrogens. This same study found a threefold increase in growth hormone (GH) which is the stimulator of IGF-I secretion. Von Puttkamer[93] and

[‡] Studies were identified by a PubMed search of the English language literature using the terms *tall* AND girl OR female AND treatment OR hormone OR *diethylstilbestrol* OR o/estradiol OR o/estrogen for any field covering all dates up to the time of writing. The reference lists of all the publications identified by this search were inspected for additional studies. The findings and characteristics of all studies that explored the association between hormone treatment with high-dose estrogens and endogenous hormone levels were reported.

colleagues suggested a negative feedback mechanism between GH and IGF-I (somatamedin) as the reason for this response.

Table 2.2: Summary of mean total plasma IGF-I (ng/ml) before, during and after ethinyl estradiol treatment for tall stature reported in five studies.

Study	N	Dose of Estrogen mg/day	IGF-I before treatment ng/ml	IGF-I during treatment ng/ml		IGF-I after treatment ng/ml	
				0–6 months	6–12 months	0–6 months	6–12 months
Svan et al. (1991)[11]	21	1.0	455	200			222
	20	0.50	411	178			211
	15	0.25	400	311			277
Rooman et al. (2002)[10]	16	0.1	528	376		-	-
	8					243	354
Rooman et al. (2005)[12]	19	0.1	530	350		-	-
	18					258	450
Gourmelen et al. (1984)[91]	13	0.25–0.3	1.64*		1.34* † 1.39* 0.99*	1.02*	
Wajs-Kuto et al. (1999)[13]	22	0.1	413	327	302		
	36	0.2	478	348	253		

* Micromole per ml (U/ml)

† Results at 6, 12 and 18 months, respectively.

Most of the studies in **Table 2.2** reveal lower post-treatment levels of IGF-I compared to baseline. It has been suggested that this reduction may be due to the age-dependent decrease in IGF-I typically seen at the end of puberty[10].

As discussed earlier, the observed reduction in IGF-I levels is believed to be the mechanism by which estrogen promotes bone maturation and subsequently, reduces final height.

IGF-I binding proteins

Three studies have examined the effect of estrogen treatment for tall stature on IGF binding proteins. IGF binding proteins (IGFBP 1-6) determine the pharmacokinetics of IGF-I, which in turn determines its overall bio-availability in the tissues. For example, IGF-I binds to IGFBP-3 that together with an acid-labile subunit, forms a ternary complex that cannot cross the vascular endothelium thus affecting its bioavailability to tissues[12] and prolonging its presence in the circulatory system.

Rooman and colleagues observed a reduction in circulating IGFBP-2 and an increase in IGFBP-1 & 4 with treatment[10]. The change in IGFBP-2 & 4 was repeated in a later study with a larger group of treated girls. The significance of IGFBP-2 in relation to IGF-I is unknown, though a study that examined the animal model of the role of IGFBP-2 in long bone growth, suggests it to have an inhibitory effect on IGF-I[94]. IGFBP-4 is known to inhibit IGF-1, and it has been suggested that the increase in this binding protein mediates the growth inhibiting effect of high-dose estrogens[12].

Rooman and colleagues[12] also observed a reduction in the levels of IGFBP-3, but only at the end of therapy, suggesting a reduction only with long-term use. Estrogen did not appear to have an effect on IGFBP-5 in either study. IGFBP-6 decreased during estrogen administration and increased after therapy. The significance of these changes is not yet clearly understood.

Despite these modulations of the IGF binding proteins, the changes are not substantial[10], particularly in relation to IGFBP-3, the main binding protein present in serum[13]. Rooman and colleagues[10] suggested that the estrogen induced changes in IGF-I, observed with treatment in girls, is more likely to be due to a direct inhibitory action of estrogen on IGF-I synthesis rather than through any modulating effect on IGF binding proteins.

IGF-II competes for the same binding sites on the IGF-I binding proteins resulting in a rise when IGF-I drops and vice versa[12]. High-dose estrogen treatment in

girls has been shown to produce a slight increase in IGF-II, from a mean 407(186–612) ng/ml to 471(363–693) ng/ml in the first three months of treatment[12].

2.4.2.3 DHEA-S & gonadotropins

Dehydroepiandroeterone sulphate (DHEA-S) is the sulphated form of a precursor to androstenedione which can undergo further conversion to produce testosterone, estrone and estradiol. DHEA-S has been shown to reduce by 40% in girls treated with ethinyl estradiol (0.1 mg and 0.2 mg/d)[13]. According to Wajs-Kuto et al. (1999)[13], this is consistent with the effect observed with lower doses of ethinyl estradiol contained in the oral contraceptive pill.

The gonadotropins (LH and FSH) have also been shown to be reduced in girls treated with high-dose estrogens[16]. These changes could have implications for pubertal development. Messinis (2006)[89] summarised the involvement of these gonadotropins during the pubertal period in normal girls. In pre-pubertal girls, LH and FSH levels are generally very low due to the suppression of gonadotropin-releasing hormone (GnRH) from the hypothalamus[89]. During puberty, the hypothalamus-pituitary system is activated and LH and FSH levels are increased, in a pulsatile manner[89]. Circulating levels of these gonadotropins increase gradually as puberty progresses, with early lower levels stimulating follicle maturation and estrogen synthesis in the ovaries. The higher levels of circulating estrogen subsequently stimulate proliferation of the endometrium that leads to the first menstruation. This effect of estrogen on the endometrium is observed during treatment with high-dose estrogens for tall stature. Menses occurs within three months of treatment in girls who are pre-menarchial at start of treatment.

In normal pubertal girls, even as late as Tanner Stage 5, the estrogen levels (~60 pg/ml) are generally not high enough to exert a positive feedback mechanism on GnRH to increase the LH and FSH concentrations to a level sufficient enough to stimulate ovulation[89]. Consequently, there is a lengthy period of anovulation after menarche as discussed previously. Menstrual periods are generally irregular during this anovulatory period, and it is not until progesterone is produced from the mature corpus luteum during

the ovulatory period, that menses become regular. This explains the need for a progestagen a few days a month in girls treated with high-dose estrogens to promote regular cyclic bleeding.

Complete suppression of gonadotropin secretion (e.g LH, FSH) with high-dose estrogen therapy in tall girls has been observed and, according to Commentz and Willig[16], expected, as it is an indicator of good treatment compliance. This suggests that girls undergoing therapy with high-dose estrogens are anovulatory despite the occurrence of menses.

It may be the addition of a progestagen to the regimen, in order to induce cyclical bleeding, that contributes to the reduction in gonadotropins. In the oral contraceptive pill, adding a progestagen to the estrogen regimen appears to produce greater suppression of plasma gonadotropins than estrogen by itself[95].

An increase in cortisol has also been observed[16]. This increase is also expected because of estrogen's well established augmentation of cortisol-binding-globulin (CBG) production[16].

2.4.2.4 Prolactin

Estrogen treatment for tall stature has also been shown to increase prolactin levels[13, 19, 87]. One study measured prolactin concentrations before and one, three, six and 12 months after commencement of treatment for two doses of estrogen (0.1 and 0.2 mg/day). **Table 2.3** presents the changes in prolactin levels compared with baseline levels. While the greatest change in prolactin levels occurred after three months of treatment, prolactin levels remained greater than baseline levels at least 12 months into treatment. However, caution is required when interpreting these findings because of the large variability in prolactin concentrations (as demonstrated by the size of the standard deviations).

Table 2.3: Changes in prolactin concentration (mean ± SD) during ethinyl estradiol treatment for tall stature compared with values before treatment (μg/ml).

Study	N	Dose of Estrogen mg/day	Prolactin before treatment μU/ml	Prolactin during treatment Δ (μU/ml)		
				3 months	6 months	12 months
Wajs-Kuto et al. (1999)[13]	15	0.1	183±50	Δ+229±213	Δ+136±112	Δ+81±84
	20	0.2	198±86	Δ+375±252	Δ+226±132	Δ+182±176

Hanker et al.(1979)[87] (not in table) measured prolactin levels soon after estrogen therapy in girls. Prolactin levels >20 ng/ml (exceeding normal range) were found in eight of 14 girls, although these levels were only temporary, all reducing to normal levels within eight weeks[87]. In contrast, Houdijk et al. (2000)[19], observed sustained mean increases 12 months into treatment in 37 girls, treated with 0.2 mg/day of ethinyl estradiol. Mean prolactin levels were 180 μU/ml before treatment, and 450, 400 and 350 μU/ml at 3, 6 and 12 months into treatment, respectively. Prolactin levels dropped back to pre-treatment levels 0–6 months following cessation of treatment (170 μU/ml).

A case-study of a girl treated with 0.3 mg/d of ethinyl estradiol reported a serum prolactin concentration of 58.2 ng/ml, four days after cessation of treatment. This is compared with 1.7 ng/ml measured at start of treatment (normal <20 ng/ml). A CT scan of the brain identified a pituitary tumour, which one year later was reduced by 20–30% with bromocriptine therapy[18]. The authors stated that estrogen stimulation of a pre-existing tumour could not be excluded.

Consistent with these findings is the observation in a cross-sectional study of an increase in serum prolactin concentrations in women using the contraceptive pill (containing ethinyl estradiol), with higher doses (i.e. 50 μU/day) eliciting a larger increase[96]. This is in contrast to a study that examined the effects of the oral

contraceptive pill on hormonal metastasis in adolescents (12–17 years)[97]. No change in prolactin levels were observed six or 12 months into therapy, compared with baseline levels. However, only 23 and 13 of the 46 recruited girls provided blood measures at six months and 12 months respectively.

The effect of treatment with high-dose estrogens on the levels of these endogenous hormones may be important to breast physiological and disease outcomes. Many of these hormones have been associated with breast histology, function and disease (e.g. estrogen, progesterone, IGF-I and prolactin). These associations are reviewed in Chapters 3 and 6 in relation to breast disease and function, and mammographic density, respectively.

2.4.3 Adverse effects of treatment

Side effects of treatment with high-dose estrogens have been reported in the research literature[§]. This section describes the short- and long-term side effects separately.

2.4.3.1 Short-term side effects of treatment

A number of short-term side effects of treatment have been reported in the literature, many of these breast related. The breast related side effects reported in the literature include breast pain[22], pigmentation of the areolae and nipple [22, 23, 29, 43], gallactorrhoea[23, 43] and more rarely, benign breast disease[23, 29]. A detailed review of the short-term effects on the breast is presented in Chapter 3.

The common non-breast related side effects include headache, nausea, weight gain and leg cramps. Less common effects of treatment include thrombosis, endometrial polyps or hyperplasia, ovarian cysts, and menstrual bleeding disturbances. A summary of case-series and follow-up studies reporting these side effects is provided in **Table 2.4** below.

[§] See footnote p26 for method of identification of studies. All studies identified this way that explored and reported on the side effects of treatment were reported.

Table 2.4: Summary of studies reporting non-breast related short-term side effects of treatment with high-dose estrogens in adolescent girls.

Study	N	Estrogen type	Dose mg/day	Type of study	Side effect during treatment
Kuhn et al. (1977)[43]	36	EE	0.5	Case-series (reports at time of treatment)	Weight gain: "almost all girls", mean 11.1 kg Nausea "most girls" and vomiting Migraine (n=1)
Trygstad (1986)[23]	680	DES & P EE & P EE & P Other formulations or doses	5.0 0.5 0.25 0.100	Case-series (reports at time of treatment)	Weight gain (90%) Nausea (60%) Migraine (2%) Vaginal fluid (2%) Increased myopy (2%) Interval bleedings (2%) Endometrial hyperplasia (n=1) (no progestagen)
de Waal et al. (1995)[29]	180	EE	0.100–0.200	Follow-up of patients (mean 10 years post-treatment).	Weight gain (41%) Nausea (14%) Migraine (13%) Vaginal discharge (13%) Leg cramps at night (20%) Interval bleeding (6%) Cysts/tumours of uterus/ovaries (1%)
Weimann et al. (1998)[22]	50	Conjugated	7.5–11.25	Follow-up of patients (up to 6 years post-treatment)	Weight gain (>10 kg) (70%) Nausea (10%) Headache (14%) Calf cramps (6%) Abdominal pain (6%) Oedema (6%) Allergic skin reaction (6%) Hypertension (2%) Dysuria (6%)Hyperihernia (6%)

Study	N	Estrogen type	Dose mg/day	Type of study	Side effect during treatment
Crawford (1978)[41]	130	DES & P (104) EE & P (26)	5.0 0.25	Case-series (reports at time of treatment	Weight gain Nausea (morning) Migraine (n=3) Night cramps ('generally familiar') Vaginal discharge Intermenstrual bleeding (10%) Hypertension (37%) Urticaria (n=2)
Radivojevic et al. (2006)[48]	26	17β-estradiol	4.0–8.0*	Case-series (reports at time of treatment)	Fatigue Nausea Abdominal discomfort Triglyceride discomfort
Binder et al. (1997)[85]	56	Conjugated estrogens	7.5 mg	Follow-up (~10 years)	Weight gain (13.1%) Calf cramps (17.2%) Orthostatic problems (7.1%)
Conte et al. (1978)[51]	904	Conjugated estrogens Ethinyl estradiol DES Estradiol	Mixed	Follow-up via treating endocrinologists (retrospective, time not reported)	Weight gain (69%) Nausea (48%) Leg cramps (3%) Irregular menses (14%) Hypertension (3%) Polyps or endometrial hyperplasia (0.3%) Ovarian cysts (0.3%) Thromboembolism (0.1%) Glucose intolerance (0.2%)

EE=ethinyl estradiol
DES=diethylstilbestrol
P=progestagen
*8.0 mg of 17β-estradiol is bioequivalent to 0.100 mg of ethinyl estradiol (EE)

In addition to the above studies, a survey of US paediatric endocrinologists by Barnard et al. (2002)[3] revealed 86% had patients who had experienced weight gain, 77% nausea or vomiting, 74% headache/migraine, 49% irregular menses, 37% polyphagia, 37% leg cramps, 17% dizziness/orthostatic problems, 16% polyps or endometrial hyperplasia, 23% hypertension, 16% ovarian cysts, 16% thrombosis and 15% glucose intolerance (either commonly, occasionally or rarely).

2.4.3.2 Long-term effects on reproductive tissue

No studies have examined the long-term effects of treatment on breast tissue though the long-term effects on a range of reproductive outcomes have been reported. De Waal et al. (1995)[29] described menstrual characteristics and reproductive outcomes for treated and untreated women in the Netherlands. No effect on reproductive outcomes was observed but the sample size was small and the average follow-up was only 10 years after treatment (mean age 25 years).

However, the longer follow-up study of Australian tall girls described earlier, found treatment with high-dose estrogens in adolescent to have long-term effects on fertility[25], suggesting longer term effects on reproductive tissue. In this study 1432 eligible individuals (mean age 39 years) were identified from medical records of Australian paediatricians who assessed or treated tall girls from 1959 to 1993, and from self-referrals. Women whose parent had sought a medical opinion about their tall stature and who had had a radiological assessment of their skeletal age were eligible to participate. They included girls who had received estrogen treatment (3 mg DES daily or 150 μg EE daily) in adolescence to reduce their adult height (treated group) and those who had not (untreated). Treated (n=371) and untreated (n=409) women completed interviews about reproductive history, fertility problems and sexual history[25]. After adjustment for age, treated women were more likely to have ever tried for 12 months or more to become pregnant without success (RR 1.80, 95% CI: 1.40 to 2.30); more likely to have seen a doctor because they were having difficulty becoming pregnant (RR 1.80, 95% CI: 1.39 to 2.32); and more likely to have ever taken fertility drugs (RR 2.05, 95% CI: 1.39 to 3.04)[25]. Time to first pregnancy analysis showed that the treated group was 40% less likely to conceive in any given menstrual cycle of unprotected

intercourse (fecundability ratio 0.59, 95% CI: 0.46 to 0.76)[25]. An obvious cause of the fertility problems could not be readily identified (sexual history was not found to be a confounder) but an overall excess of endometriosis and ectopic pregnancies in the treated tall girls compared with untreated tall girls suggested that treatment may have affected the developing reproductive tract.

2.5 Overview

This chapter outlined the use of treatment to reduce final height in tall girls and the effectiveness of treatment in relation to final height reduction and improved psychosocial outcomes.

While available evidence strongly suggests that treatment is effective at reducing final height in tall girls, the degree of height reduction between studies ranges between 2.1 to 10.0 cms. High variability in the the level of effectiveness between the studies is due to differences in the height prediction methods used, treatment regimens, and timing of treatment between studies[44]. The study of Australian tall girls which matched treated and untreated women on their estimated mature height (EMH) and adjusted for error in EMH predictions observed an unadjusted treatment effect of minus 2.5 cm[77].

While psychosocial factors are often cited as the main reason for treating tall stature, there is no clear evidence that treatment improves psychosocial outcomes. Apart from anecdotal observation from treating physicians, only two studies had formally investigated the psychosocial outcomes of treatment. These retrospective studies revealed no differences between the two groups in a range of psychosocial outcomes[84 54].

This chapter identified studies that had shown treatment to modify levels of some endogenous hormones. These modifications include the suppression of insulin-like growth factor-I (IGF-I)[10-13, 91], dehydroepiandrosterone sulphate (DHEA-S)[13], testosterone[13] and basal and GnRH stimulated gonadotropins[16]; and increased prolactin[13, 18, 19], IGF-II[12] and cortisol[13, 14] levels. While there is consistency in the findings between these longitudinal studies, some

degree of caution is required when interpreting these findings. The sample sizes were small (ranging from 8-36), and none used control groups.

The above review examined Australian and international published case-series and follow-up reports of short-term side effects of treatment in tall girls. Four case-series reports by treating specialists, and eight follow-up studies (ranging from 6-10 years post treatment) desribed short-term side effects of treatment that include headache, nausea, weight gain and leg cramps. Less common effects of treatment include thrombosis, endometrial polyps or hyperplasia, ovarian cysts, and menstrual bleeding disturbances. There are a number of limitations to these studies. The prevalence of effects by treatment type has not been reported, nor have all side effects been examined consistently across studies. Most of the case-series reports were based on small sample sizes, and the outcomes were selected by the treating physicians rather than independent researchers. For uncommon outcomes, it is unclear the degree to which physicians systematically examined girls for these conditions in the studies, and subsequently reported them.

While, no studies have examined the long-term effects of treatment on breast tissue, the long-term effects on a range of reproductive outcomes have been reported. One study observed no effect on reproductive outcomes but the sample size was small and the average follow-up was only 10 years after treatment (mean age 25 years). A longer follow-up study of Australian tall girls found treatment with high-dose estrogens in adolescence to have long-term effects on fertility[25], suggesting longer term effects on reproductive tissue.

These findings, together with the hormone changes and short-term side effects on the breast described in this chapter, suggest that high-dose estrogen treatment for tall stature in girls may have longer term effects on breast histology and function.

Box 2.1: Key points from the literature in Chapter 2.

KEY POINTS FROM THE LITERATURE: CHAPTER 2

- Tall girls have been treated with high-dose estrogens for psychosocial reasons since the 1950s. The practice of using high-dose estrogens to reduce height in adolescent girls is uncommon now.
- In Australia girls were typically treated with diethylstilbestrol up to 1971 and ethinyl estradiol after this time. A progestagen was typically added to the regimen 4–5 days a month to induce cyclical bleeding.
- Estrogen works by fusing the growth plate in long bones. The mechanism of action is still not clear.
- Studies have reported estimates of height reduction ranging from 2.1 to 10 cm in treated girls.
- There is no clear evidence that treatment improved psychosocial outcomes.
- Treatment with high-dose estrogens appears to modify the levels of some endogenous hormones.
- Short-term side effects of treatment include headache, nausea, weight gain and leg cramps. Less common effects of treatment include thrombosis, endometrial polyps or hyperplasia, ovarian cysts, and menstrual bleeding disturbances.
- Long-term effects on fertility have been observed in the Australian study of tall girls.

PART B

Chapter 3: Current evidence on the short- and longer term effects of estrogen treatment in adolescence on breast symptoms, disease and function

Chapter 4: Treatment with high-dose estrogens in adolescence: short-term effects on the breast and longer term breast disease

Chapter 5: Treatment with high-dose estrogens in adolescence and subsequent effects on lactation

3: CURRENT EVIDENCE ON THE SHORT- AND LONGER TERM EFFECTS OF ESTROGEN TREATMENT IN ADOLESCENCE ON BREAST SYMPTOMS, DISEASE AND FUNCTION

3.0 Introduction

This chapter reviews Australian and international published case-series and follow-up reports of adverse effects on the breast during and shortly following cessation of estrogen treatment in tall girls. The review highlights the gaps in our understanding of the short- and long-term effects on the breast and the importance of this PhD research in narrowing this gap. This chapter then follows with a review of published epidemiological, molecular, *in vitro* and *in vivo* studies that have examined associations between estrogen exposures and breast disease. The evidence presented supports the suggestion that high-dose estrogen treatment for tall stature in adolescent girls may increase the risk of developing breast disease later in life. As further support, key stages in the development of the mammary gland are briefly summarised to highlight the importance of adolescent mammary development to adult breast health and function.

This chapter then finishes with an exploration of the evidence around the postulate that high-dose estrogen exposure during adolescence may affect subsequent breast function, or more specifically, lactation. It presents the few available epidemiological and animal studies that have investigated the effect of exogenous estrogen exposures on lactation. When findings from these studies are considered together with the reported changes in the hormonal milieu of girls treated with estrogens described in Chapter 2, and previous findings from the Australian Tall Girls Study on the long-term effects of treatment on fertility, it seems possible that treatment could have long-term effects on breast function. It is also evident that no other study has examined the long-term effect of high-dose estrogen treatment in adolescent girls on mammary function later in life.

3.1 Short-term side effects on the breast

A number of breast related short-term side effects of treatment with high-dose estrogens in adolescent girls have been reported and include galactorrhea, increased pigmentation of the areolae and nipples, breast pain and, more rarely, benign breast disease (e.g fibroadenoma)**. Case-series reports and follow-up studies that describe short-term side effects of treatment for tall stature on the breast are reported below.

3.1.1 Galactorrhea

Galactorrhea is a condition characterised by the milky discharge from one or both nipples not related to breastfeeding and has been reported to be a side effect of treatment with high-dose estrogens in adolescent girls. The frequency and potential mechanism for this adverse effect is described below.

3.1.1.1 Frequency of side effect

Of four case-series and two follow-up studies (see **Table 3.1**) the prevalence of galactorrhea as a side effect of treatment for tall stature ranges from 0 to 14%[43]. Two additional studies surveyed endocrinologists asking them how frequently particular side effects occurred in female patients that they treated. In the first of these studies, none of the 77 US or European endocrinologists surveyed (904 patients combined) revealed galactorrhea to be a side effect of treatment[51]. The patients of the surveyed specialists were treated predominantly with ethinyl estradiol or conjugated estrogens. Of 82 US endocrinologists surveyed in the second study, none said galactorrhea was common among their patients, one said they came across it occasionally, 13 (16%) rarely, while 68 (83%) reported that they had never come across the side effect among their treated patients[3]. It is unclear why the frequency of galactorrhea as a

** See footnote p26 for method of identification of studies. All studies identified this way that explored and reported on the side effects of treatment on the breast were reported.

side effect of treatment varies widely between studies. Treatment type or dose may explain these differences. These studies do not differentiate between treatment types. In addition, some degree of random variation in prevalence is expected given the small sample sizes and uncommon outcomes.

3.1.1.2 Mechanism of treatment induced galactorrhea

Treatment induced galactorrhea is likely to be due to the increased prolactin levels that occurs in treated tall girls[13]. It is not clear whether the reports above refer to galactorrhea during treatment or galactorrhea following cessation of treatment. According to a review by Chatterton[98], withdrawal of estrogen, particularly if prolactin levels remain elevated, may precipitate galactorrhea.

3.1.2 Increased pigmentation of the areolae and nipples

According to published reports, increased pigmentation of the areolae and nipples is the most reported breast related side effect of estrogen treatment for tall stature in adolescent girls. The reported frequency and potential mechanism of these observed effects is described below.

3.1.2.1 Frequency of side effect

Of the studies described in **Table 3.1** that examined breast related side effects of treatment, the prevalence of increased pigmentation of the areolae and nipples ranged between 3%[22] to 38%[99] [††]. The survey by Barnard et al. (2002)[3] of US endocrinologists (not in table) who had treated adolescent girls with high-dose estrogens found that 72% had observed this side effect in one or more of their patients[3]. Of 86 respondents, 12 (14%) stated that increased pigmentation was common among their patients, 26 (30%) said they came across it occasionally, 24 (28%) rarely, and 24 (28%) never[3]. The separate survey of US and European endocrinologists referred to above (Section 3.1.1.1) did not report increased pigmentation of the nipples and areolae as a side effect of treatment[51].

[††] German language article cited by Drop 1998

Table 3.1: Breast related side effects of high-dose estrogen treatment for tall stature in adolescent girls.

Study	N	Estrogen Type*	Dose mg/day	Type of study	Side Effect (breast related)
Kuhn et al. (1977)[43]	36	EE	0.5	Case-series	Increased pigmentation of areolae and nipples ("in many") Galactorrhea during therapy (14%).
Trygstad (1986)[23]	680	DES & P EE & P EE & P Other formulations/doses	5.0 0.5 0.25 0.100	Case-series	Pigmentation of areolae and nipples (23%) Galactorrhea (2%) Benign breast fibroadenoma (n=2, 0.3%) Flat-chestedness (n=7)
de Waal et al. (1995)[29]	180	EE	0.100–0.200	Follow-up of patients (mean 10 years post-treatment)	Pigmentation of areolae and nipples (27%) Galactorrhea (4%) Breast cysts/tumours (1%)
Weimann et al. (1988)[22]	50	Conjugated	7.5–11.25	Follow-up of patients (up to 6 years post-treatment)	Breast discomfort (6%) Increased pigmentation (2%)
Crawford (1978)[41]	130	DES & P (104) EE & P (26)	5.0 0.25	Case-series during treatment	Flat-chestedness (% not reported) Increased pigmentation of the areolae and nipples (% not reported) Benign intraductal papilloma (EE) (n=1)
Radivojevic et al. (2006)[48]	26	17β-estradiol	4.0–8.00 †	Case-series during treatment	Breast discomfort (n=1) Increased pigmentation (part of body not specified)

* EE=ethinyl estradiol, DES=diethylstilboestrol, P= progestagen

† 8.0 mg is bioequivalent to 0.100 mg of ethinyl estradiol (EE)

3.1.2.2 Mechanism of treatment induced pigmentation of areolae and nipples

Darkened pigmentation, a common occurrence in pregnancy, is believed to be due to high levels of circulating estrogen[100]. Darkened areolae in newborn girls is an indicator of estrogen excess[100]. This effect on the breast has also been reported to occur following the accidental exposure to estrogen in pre-adolescent girls. In 1953, Cook, McArthur & Berenberg[101], reported a darkening of the pigmentation of the nipples and areolae and growth of the breast in girls aged four and seven following the accidental ingestion of estrogen tablets.

3.1.2.3 Side effects by treatment type

Reports suggest that the severity of the increased pigmentation in girls treated with estrogens for tall stature depends on the form of estrogen used. According to Drop[44], the majority of 40 treated girls in Zackman and colleagues' case-series report[46] had experienced darkening of the areolae, which was more marked in the DES treated girls than the EE treated girls. Wettenhall and Roche (1965)[52] described the common occurrence of darkening of the areolae with treatment using diethylstilbestrol which did not fade completely on cessation of treatment. In contrast, a decade later, Wettenhall stated that pigmentation always faded and skin returned to its normal colour some months after treatment cessation[60].

The more pronounced effect of DES on pigmentation of the nipple and areolae was also observed by Crawford[41] (p 1,193):

> *" When diethylstilbestrol is given, pigmentation of the nipples, areolae, linea alba, and skin creases of the neck are predictable side effects in girls who are inherently good tanners... When ethinyl estradiol is used, the enhancement of pigmentation is so minor that it scarcely merits discussion with the child before therapy is begun".*

None of the reports described above provide prevalence of the side effect by treatment type.

3.1.3 Breast hypoplasia

Breast hypoplasia or micromastia is the incomplete or under-development of the breast and has been reported to be a side effect of treatment with high-dose estrogens for the treatment of tall stature in adolescent girls as described below.

3.1.3.1 Frequency of side effect

According to Crawford, one of three principal reproductive organ complaints to arise in follow-up studies (references not provided) of treated patients at Massachusetts General Hospital was 'flat-chestedness'[41]. The number within the Massachusetts cohort (104 DES, 26 EE treated) who reported this problem was not presented. The only other published report of under-developed breasts as a side effect of estrogen treatment was made by Trygstad[23]. In this report, complaints of under-development were made by seven of a total 680 treated tall girls (see **Table 3.1**). These reports of flat-chestedness were made by the girls themselves. It is unclear whether girls noticed a reduction in breast size with treatment or perceived treatment to have reduced the growth velocity of their breasts. There was no clinical assessment to verify these reports.

An interview of a Danish endocrinologist who recently treated girls for tall stature was published in a prominent Australian newspaper (2007)[55]. The endocrinologist was asked by a journalist whether treatment was likely to 'flatten the figure' as suggested by a tall girl (also interviewed by the journalist) who had friends who had been treated. The endocrinologist said that it was possibly a side effect of treatment but that the long-term adverse effects on the breast were not known.

3.1.3.2 Mechanisms of treatment induced breast hypoplasia

Under-development of the breast (hypoplasia or micromastia) can have a congenital aetiology (e.g. ulnar-mammary syndrome, Poland's syndrome, Turner's syndrome, and congenital adrenal hyperplasia); or it can be acquired (e.g. due to trauma[102] or radiotherapy of

the prepubertal breast-bud[103])[104]. Another explanation is provided in a study by Pertzelan (1982)[105] who examined breast development (according to Tanner stages–see Chapter 2, Section 2.4.1.1) in girls treated with estrogen for estrogen deficiency and under-developed breasts. In this study, 45 girls were placed into four groups: those with gonadal dysgenesis, isolated gonadotropin deficiency, multiple pituitary hormone deficiencies, and congenital adrenal hyperplasia. The patients with gonadal dysgenesis, and a normal functioning hypothalamic-pituitary, achieved full breast development after estrogen treatment. In contrast, those with isolated gonadotropin deficiency and multiple pituitary hormone deficiency displayed incomplete breast development even after three or more years of estrogen treatment. The authors of the study highlighted the major difference in hormonal status between those achieving full breast development and those who did not. The latter groups that did not achieve complete breast development lacked gonadotropins. This led the researchers to suggest that gonadotropins had an important role in mammary gland development[105]. These findings are interesting because, as reported in Chapter 2, gonadotropins (LH and FSH) were suppressed with treatment for tall stature[16]. It is possible that insufficient gonadotropin levels during treatment contributed to incomplete breast development.

Another possible explanation for under-developed breasts in treated girls may be related to IGF-I, a growth factor believed to play an important role in mammary gland development[20, 21]. It is possible that the reduction in IGF-I levels observed with treatment (see Chapter 2, Section 2.4.2.2), adversely affects mammary gland development.

Treatment has been reported to accelerate the development of the breasts[46]. In one report, accelerated pubertal development has been observed in up to 90% of treated girls[23]. Whether this acceleration results in less overall growth of the breast is unknown.

3.1.4 Breast pain

Breast pain, or mastalgia, not related to lactation, can have a number of causes. Preece and colleagues (1976)[106] identified six specific aetiologies for breast pain, excluding causes of

pain arising outside of the breast and those of unknown aetiology. The aetiologies include cyclical pronounced mastalgia (hormone related), duct ectasia (blocked milk duct), Tietze syndrome (inflammation of one or more of the costal cartilages), trauma, sclerosing adenosis (benign cellular proliferation of the lobule), and cancer[106]. Other causes not included by Preece, particularly for moderate/severe pain for >6 months include: fibroadenoma, cysts, and duct papilloma[107]. Severe cyclical mastalgia has been associated with breast cancer susceptibility[108-110].

3.1.4.1 Frequency of side effect

According to Weimann et al. (1998)[22], 6% of 50 girls treated with high-dose estrogens reported breast discomfort during treatment with conjugated estrogens[22] (**Table 3.1**). In a separate cohort[48], one of 26 girls treated with 17β estradiol was reported to have experienced breast discomfort. No other study described in **Table 3.1** reported breast pain as a side effect of treatment. Most of the case-series reports were based on small sample sizes, and the outcomes were selected by the treating physicians rather than independent researchers. For uncommon outcomes, it is unclear the degree to which physicians systematically examined girls for these conditions in the studies, and subsequently reported them.

3.2 Benign breast disease

Benign breast disease has been reported in the literature to be a rare side effect of treatment. A description of the different types of benign breast diseases and the reported prevalence of this disease in treated girls is described below.

3.2.1 Definitions and types of benign breast disease

Benign breast disease encompasses a large number of benign breast abnormalities and has many classifications. According to the ANDI classification (Aberration of Normal Development and Involution), benign breast diseases include: giant or multiple fibroadenomas as an extension of aberrant lobular development (age of occurrence 15–25

years); incapacitating mastalgia as an extension of aberrant responses to cyclical changes during menstruation; periductal mastitis as an extension of aberrant lobular involution; and epithelial hyperplasia with atypia as an extension of aberrant epithelial turnover[111] (See **Figure 3.1**).

Figure 3.1: Aberration of Normal Development and Involution (ANDI)[111] classification of benign breast diseases.

ANDI Classification

Benign Breast Disease		Cause
Epithelial hyperplasia with atypia	→	Aberrant epithelial turnover
Giant/Multiple fibroadenoma	→	Aberrant lobular development
Severe mastalgia	→	Aberrant response to cyclical changes during menstruation
Periductal mastitis	→	Aberrant lobular involution

Benign breast diseases have also been classified by Dupont and Page (1985)[112] as three main histological types: proliferative benign breast disease, with or without atypical ductal or lobular hyperplasia; or non-proliferative benign breast disease. Non-proliferative benign lesions of the breast include cysts, papillary changes, epithelial calcifications, mild hyperplasias and fibroadenomas[113]. Benign breast diseases have also been classified within two broad categories: fibroadenomas (benign tumours), and fibrocystic disease[113]. Histologically, abnormalities of fibrocystic breast disease are of epithelial origin, while those of fibroadenomas originate in the lobules[113]. Fibrocystic disease is also commonly referred to as: cystic hyperplasia, cystic disease, or epithelial dysplasia[113]. The latter classification system seems to be used in studies reporting breast related side effects of girls treated with high-dose estrogens.

3.2.2 Frequency of benign breast disease in treated girls

Benign breast disease has been reported as a side effect in treated girls[29] although reports are less common than for the other breast related side effects (**Table 3.1**). Two cases of fibroadenoma were diagnosed in a case-series of 680 treated girls (Trygstad, 1986)[23] after one year of DES treatment. Benign breast cysts/tumours were also reported to occur in 1% of 180 treated girls[29].

Conte et al. (1978)[51] surveyed members of two endocrine societies for their views and practices with respect to the use of estrogens in children and adolescents and asked about the side effects experienced by the patients of treating endocrinologists (n=77). Of 904 patients treated with estrogen, cystic hyperplasia of the breast was reported as a side effect of treatment in eight (0.1%).

The survey of US paediatric endocrinologists[3] by Barnard et al. (2002)[3] revealed 17% of treating endocrinologists had observed cystic hyperplasia of the breast in one or more of their patients as a side effect of treatment. Of 81 responders, three had occasionally observed the side effect in their patients, 11 rarely and 67 never. It is unclear, whether or not; treating endocrinologists were asked to report on the occurrence of fibroadenomas as a side effect in their patients.

3.2.3 Estrogen and benign breast disease

It has been suggested that high levels of circulating estrogen is the cause of proliferative benign breast disease[114] and fibroadenoma[115]. A review of the literature‡‡ identified studies reporting the association between estrogen therapy (estrogen replacement therapy, oral contraceptive pill) or abnormal estrogen/progesterone ratios and benign breast disease. While some of these exposures occurred in older women (e.g. menopausal women in relation to

‡‡ Studies were identified by PubMed search of the English language literature using the terms breast AND benign (OR disease OR disorder OR proliferative OR fibroadenoma OR hyperplasia) AND hormone (OR estrogen OR progestagen OR progesterone) for any field covering all dates up to the time of writing. The reference lists of all the publications identified by this search were inspected for additional.

estrogen replacement therapy), this review is interested in the effects on all ages. The effects of hormone exposures on benign breast disease might not be age dependent.

Prolactin and IGF-I levels, which have been shown to be modulated in some treated girls (see Chapter 2, Section 2.4.2) have also been linked to benign breast disease. A review of these studies is presented below.

3.2.3.1 Estrogen replacement therapy

A review of epidemiological studies by Silvera and Rohan (2008)[116] examined the association between HRT use and benign proliferative epithelial disorders (with and without atypia). It included findings from one cohort[117] and three case-controls studies[116, 118]. The cohort study by Rohan and Miller[117] observed a statistically significant association between benign proliferative epithelial disorders of the breast (BPED) and greater than eight years of HRT use in postmenopausal women (incidence rate ratio IRR 1.70, 95% CI: 1.06 to 2.72), while one nested case-control study[118] and two case-control studies[119, 120] observed no association between "ever using HRT" and benign proliferative disease. One of these non-significant studies (Berkowitz et al. 1984[120]) observed a non-statistically significant association between HRT use ≥5 years versus never used: OR 3.0 (95% CI: 0.5 to 17.5). With such wide confidence intervals it is possible that the sample sizes for the HRT duration sub-categories examined were insufficient to demonstrate statistical significance. The sample size of the cohort studied by Rohan and Miller (1999)[117] was much larger [total n=6,134 compared to 1,608 for Berkowitz et al. (1984)[120]].

An examination of the research literature identified three case-control studies not included in the review above and a randomised controlled trial published after the review. One case-control study by Pastides and colleagues[121] reported a twofold risk of having had fibrocystic breast disease in postmenopausal women using estrogen replacement therapy compared with non-users (age-adjusted OR 2.0; 95% CI: 1.0 to 3.9), though this association was only observed in women who experienced natural menopause. The second case-control study[122] found significant associations between estrogen replacement therapy (conjugated estrogens) and fibrocystic disease if used 10 or more years (age-adjusted OR 5.2, 95% CI: 2.2 to 12.3)

and if the woman was currently using and had used for 5 or more years (age-adjusted OR 2.8, 95% CI: 1.2 to 6.5). The odds of having fibrocystic disease were greater in women who used 0.625 mg of conjugated estrogen compared with women who used 0.3 mg. The third case-control study[123] observed an age-adjusted OR of 1.4 (95% CI: 1.1 to 1.8) in ever users of HRT compared with non-users and an OR of 1.9 (95%CI: 1.2 to 2.9) for 15 or more years of use.

These results are supported by the randomised controlled trial undertaken by the Women's Health Initiative (WHI). This study (n=10,739) tested the effect of conjugated equine estrogen (0.625 mg/d) on risk of proliferative breast disease in postmenopausal women and found that women who used conjugated equine estrogen (CEE) (n=155) were more likely to have developed proliferative benign breast disease (HR 2.11, 95% CI: 1.58 to 2.81)[124] than untreated women (n=77) after a mean follow-up of seven years. Risk was greater for proliferative benign breast disease without atypia (HR 2.34, 95% CI: 1.71 to 3.20) compared to proliferative disease with atypia (HR 1.12, 95% CI: 0.53 to 2.40)[124].

It is important to separate those studies that explored estrogen only and estrogen and progesterone combined HRT formulations. Of the observational studies above, only the cohort study by Friedenreich et al.[118] had separately reported the effects of the combined estrogen and progestagen (E + P) combined formulation. They found no association between combined hormone therapy and proliferative breast disease (OR, 1.02, 95% CI: 0.75 to 1.39).

A second placebo-controlled randomised controlled trial undertaken by the WHI[125], (n=16,608) tested the effect of combined E + P formulations (0.625 mg/day of conjugated equine estrogen and 2.5 mg/day of medroxyprogesterone acetate). The hazard ratio for benign proliferative breast disease without atypia was 2.00 (95% CI: 1.50 to 2.66), while for atypical hyperplasia it was 0.76 (95% CI: 0.38-1.52) after a mean follow-up of 5.5 years. The risk ratios are greater for estrogen alone hormone therapy compared to the combined formulations. However, caution is required when comparing both studies because of the differences in baseline characteristics, event rates, and length of intervention and follow-up time[125].

Few studies examined the association between exogenous hormone replacement therapy and breast fibroadenomas. One published case-control study found an increased risk of fibroadenoma with ever use of estrogen replacement therapy in women 45 years and older (OR 2.83, 95% CI: 1.21 to 6.60)[126] while another observed an OR of 1.6 (95% CI: 0.8 to 3.5)[123] in postmenopausal women.

Overall evidence based on randomised double blind, placebo controlled trials suggests that risk of benign breast disease may be greater in women who have used estrogen replacement therapy, or combined estrogen and progestagen combined therapy for more than five years. This does not parallel the findings of studies examining the association between oral contraceptive use and benign breast disease as described below.

3.2.3.2 Oral contraceptive use

Silvera and Rohan, in their recent review above (2008)[116], also included epidemiological studies that had examined the association between oral contraceptive (OC) use and benign proliferative epithelial disease (BPED). Two cohort and six case-control studies were included in the review. Two of these studies, a case-control[119] and a nested case-control study[127], observed an inverse association between BPED and OC use (RR 0.35, 95% CI: 0.16 to 0.76) (IRR§§0.95, 95% CI: 0.85 to 1.07) respectively, while six other case-control studies[120, 128-132] observed no association. One of these case-control studies observed a stronger association between long-term use of oral contraceptives and fibrocystic disease in which epithelial atypia was minimal or absent compared with fibrocystic disease with epithelial atypia[131]. However another study did not find a difference in association between histologic type of fibrocystic breast disease[133].

An inverse association has similarly been observed between oral contraceptive use and fibroadenomas (only fibrocystic or BPED were explored in the review by Silvera and Rohan above). A follow-up of the Oxford Family Planning Association Study cohort, found a negative association between hospitalisation for fibroadenoma and total duration of oral

§§ The incidence rate ratio (IRR) is the ratio of incidence rates in those exposed to that of those unexposed.

contraceptive use for regimens containing lower dose ≤50 μg, 50 μg and >50μg estrogen[134]. Similarly, a randomised controlled study[135] observed a significant reduction in the width of existing fibroadenomas in women who took ethinyl estradiol (0.03 mg) and levonorgestrel for four consecutive cycles, suggesting a protective effect of oral contraceptives on fibroadenomas.

Of the associations reported above, all showed inverse relationships. However, a hospital based case-control study by Berkowitz et al. (1984)[136] that was not included in the review by Silvera and Rohan (2008)[116], found previous exposure to the oral contraceptive pill to be associated with an increased occurrence of fibrocystic disease [age adjusted OR 2.52 (95% CI: 1.33 to 4.77)] in postmenopausal women only[136]. The suggestion that this observation may be due to different doses or regimens between pre- and postmenopausal women was raised by the authors. The issue concerning different doses of the oral contraceptive pill across studies is discussed more fully below.

Of interest to this PhD study is the association between the use of the oral contraceptive pill at a young age and the risk of benign breast disease. Only one study was reported in the research literature. This Australian case-control study (Yu et al. 1992)[137] observed an increased risk of fibroadenoma in women (117 cases) who used oral contraceptives before the age of 20 when compared to population controls[137].

Different doses of estrogen contained within the oral contraceptive pill (OCs) could be a reason for the differences in association reported in women before the age of 20. The contraceptive pill in the 1960s contained high-doses of estrogen (100–150 μg) in the form of mestranol, the 3-methyl ester of ethinyl estradiol. When information about thrombotic side effects was observed in the late 1960s low-dose regimens containing ethinyl estradiol became available. The British government phased out high-dose estrogen contraceptive pills in 1969, but in the US[138] and Australia[139], women had the option of using high-dose or low-dose OCs through to the 1980s, though low-dose OCs were more popular. In 1984 low-dose estrogen content OCs accounted for approximately 85% of the US OC market[95]. It is possible that the contrasting effects observed in many OC studies are due to different doses and types of estrogen. As well as dose, estrogen type needs to be considered. Mestranol, while in higher

doses, may be less potent on a gram by gram basis compared to ethinyl estradiol in relation to its effects on the breast. Ethinyl estradiol is 1.7 times more potent than mestranol on a weight by weight basis, according to endometrial response and liver corticosteroid binding globulin production as endpoints[95]. They may have different potencies on breast tissue[140]. As stated by Goldzieher, 50 μg of mestranol is pharmokinetically bioequivalent to 35 μg of ethinyl estradiol, while physiologically, it ranges from 50 to 100% of the activity of ethinyl estradiol depending on the endpoint chosen[141]. Therefore, 150 μg of mestranol, used in the earlier oral contraceptive pill does is not equivalent to the same dose of ethinyl estradiol.

While overall evidence suggests that the risk of benign breast disease may be greater in women who have used estrogen replacement therapy, or combined estrogen and progestagen combined therapy for more than five years, this does not parallel the findings of studies that had examined the association between oral contraceptive use and benign breast disease as described above. Women who are exposed to estrogen, or estrogen and progestagen combined hormone therapies are postmenopausal while, for the oral contraceptive pill, it is pre-menopausal women. Any associations between these two therapies with benign breast disease need to consider these differences. Premenopausal women have significantly higher serum levels of estrogen and progesterone compared with postmenopausal women and these differences in hormone levels might explain the different findings reported above.

3.2.3.3 Abnormal estrogen progesterone ratio

Higher estrogen over progesterone ratios have also been associated with benign breast disease. This is of interest to this study because girls treated with high-dose estrogens typically took a progestagen 4–5 days a month to induce cyclical bleeding. One study[142] observed significantly lower plasma progesterone over estradiol ratios during the luteal phase in women with benign breast disease. Subnormal levels of progesterone have also been observed in women with benign breast lesions[142]. However, no such association was observed in a study that measured serum progesterone levels of women with cyclical breast pain and biopsied benign breast disease[143].

3.2.3.4 Exogenous estrogen in the animal model

Animal studies also suggest a link between exogenous estrogen and proliferative tissue growth. In their review of estrogen and progesterone action on breast cells Mauvis-Jarvis and colleagues, referred to the formation of cysts and epithelial overgrowth comparable to human fibrocystic disease, that occurred with the administration of high-doses of estrogen to ovarectomised female rats[144]. Likewise, in a different study, the administration of 17 beta-estradiol (E2) (human equivalent 1 mg/d) to postmenopausal macaques appeared to result in a significantly higher prevalence of hyperplasia (total and atypical) compared with controls[145].

3.2.3.5 Prolactin

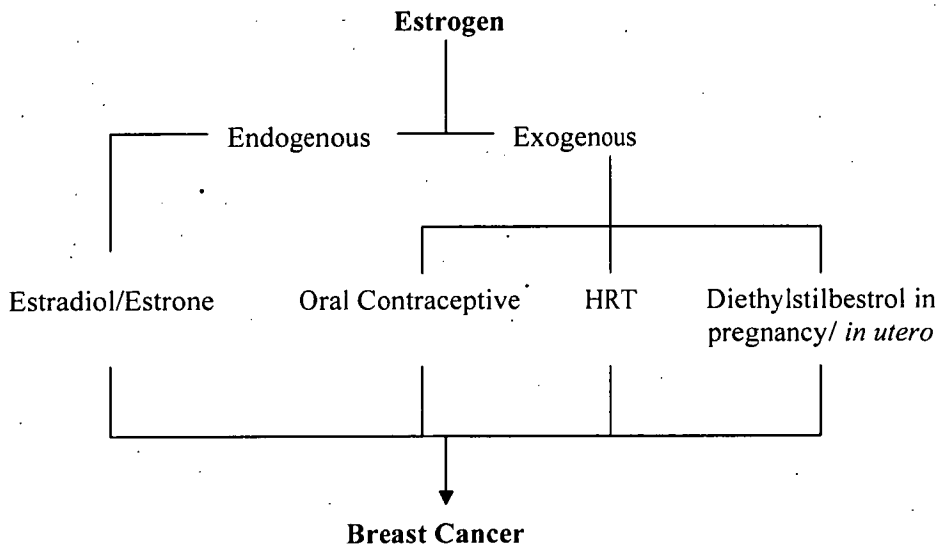
Prolactin has also been linked to benign breast disease. Girls treated with high-dose estrogen have been observed to have higher than normal prolactin levels (see Chapter 2). It is possible then, that the increased circulating prolactin observed in girls treated with high-dose estrogens for tall stature contributed to the benign breast disease observed in some published case-series reports. Local over-expression of prolactin has been associated with benign breast lesions in the mouse model that include abnormally differentiated epithelium, atrophy of the myoepithelial layer, dilated ducts, and cysts[146]. A cross-sectional study of 153 women, found that 7% (n=4) of women with operable benign breast lesions had higher than normal prolactin levels[147].

3.3 Breast cancer risk

It is plausible that high-dose estrogen treatment (ethinyl estradiol or DES) during adolescence increases the risk of breast cancer in later life. De Waal et al. (1995)[29] followed up women an average 10 years following treatment (mean age 25 years) and described menstrual characteristics and reproductive outcomes for treated and untreated women in the Netherlands. However, women in this follow-up were too young to examine breast cancer risk because breast cancer in this age group is uncommon.

A number of studies have demonstrated an association between estrogen exposures and breast cancer risk. These include studies that have examined the association between endogenous levels of estrogen (estradiol and/or estrone), or exogenous exposures (e.g. HRT, DES to prevent miscarriage in pregnant women, and the oral contraceptive pill) and breast cancer risk (**Figure 3.2**), reviewed*** in the following section. While some of these exposures occurred in older women (e.g. menopausal women in relation to estrogen replacement therapy), this review is interested in the effects on all ages. The effects of hormone exposures on breast cancer risk might be relevant to all ages.

Figure 3.2: Estrogen and breast cancer risk: studies of associations presented in the following review.



*** Studies were identified by PubMed search of the English language literature using the terms breast AND cancer AND hormone (OR o/estrogen OR estradiol OR diethylstilbestrol (DES) or contraceptive OR progestagen OR progesterone) for any field covering all dates up to the time of writing. The reference lists of all the publications identified by this search were inspected for additional studies. The findings and characteristics of all studies and/or reviews of studies that explored and reported the association between estrogen therapy or combined estrogen and progesterone therapy are described.

3.3.1 Endogenous estrogen

Associations between elevated serum and urinary levels of estrogen and risk of breast cancer have been observed. The association seems stronger in postmenopausal women as described below.

The findings of a 1997 published pooled analysis[148] of six prospective epidemiological studies suggested an association between high concentrations of endogenous estradiol and breast cancer risk in postmenopausal women. In the pooled analysis, women who subsequently developed breast cancer (n=329) had 15% (p=0.0003) higher mean concentration of blood estradiol prior to diagnosis compared with women who remained free of cancer (n=1,105)[148].

One of the researchers involved with the above published pooled analysis, later in 1999, argued that case-control studies examining the association between endogenous estrogen levels and breast cancer risk are limited by the possibility that any differences in endogenous estrogen levels observed between cases and controls, could be due to the tumour or treatment149. It was suggested that prospective cohort studies should only be examined. In 2002, the same researchers re-analysed nine prospective studies. Together these include 663 postmenopausal women who developed breast cancer two to twelve years post estradiol measurement and 1,765 who did not develop breast cancer. The RRs for women with increasing quintiles of free estradiol relative to the lowest quintile were 1.38 (95% CI: 0.94 to 2.03), 1.84 (95% CI: 1.24 to 2.74), 2.24 (95% CI: 1.53 to 3.27), and 2.58 (95% CI: 1.76 to 3.78; P for trend<0.001). These findings suggest that levels of endogenous sex hormones are strongly associated with breast cancer risk in postmenopausal women[150].

A separate and later review of epidemiological studies published in 2007 by Hankinson and Eliassen[151] concluded that a strong positive association between breast cancer risk and circulating levels of estrogens is now well confirmed among postmenopausal women. Postmenopausal women with hormone levels in the top quintile compared to the lowest were reported to have a two- to threefold higher risk of breast cancer. Evidence for

premenopausal women was reported to be inconsistent[151]. A search of the literature identified additional studies published since these reviews. The findings in these studies were also inconsistent[152-155].

While the above studies focused on serum levels of estrogen, one case-control study (n=364 breast cancer cases, 382 controls) demonstrated a positive association between elevated urinary levels of estrogen and risk of postmenopausal breast cancer[156]. The incidence rate ratio for estrone levels (highest versus lowest quartile) was 2.5 (95% CI: 1.6 to 3.8), while that for estradiol was 1.5 (95% CI: 1.0 to 2.3)[156].

According to Rinaldi et al. (2006)[157], the well established relationship of body mass index (BMI) with breast cancer in postmenopausal women could be partially explained by increased levels of endogenous estrogens. In their study, using the European Prospective Investigation in Cancer and Nutrition (EPIC) cohort, the association between BMI and breast cancer risk in postmenopausal women was substantially reduced after adjustment for serum levels of estrogen (from RR 1.11 95% CI: 0.99 to 1.25; to RR 0.99; 95% CI: 0.87 to 1.12)[157].

3.3.2 HRT

Overall evidence suggests that women who currently use, and have used HRT for at least five years are at increased risk of breast cancer[158, 159]. Estrogen (E) and progestagen (P) formulations appear to be associated with an elevated BC risk[160]. The histological type of breast cancer may also differ with HRT type, with risk of lobular carcinoma being greater than the risk of ductal carcinoma[161, 162], particularly for E + P formulations. Studies also support a reversible effect on risk if HRT has been discontinued for five or more years[158].

The Women's Health Initiative (2002)[159] was a randomised controlled trial, and one of three large studies to have observed an increased risk of invasive breast cancer and use of HRT. In this study (8,506 treated, 8,102 placebo) an increased risk of invasive breast cancer was observed with combined E + P HRT use after a five year follow-up: hazard ratio 1.26 (95% CI: 1.00 to 1.59), and an absolute risk of eight extra invasive breast cancers per 10,000 person-years[159]. This increased risk was not observed with the estrogen only HRT arm of the

trial, in women who had had a hysterectomy[163]. The UK Million Women cohort study (2003)[164] observed an increased risk of invasive breast cancer in women currently using estrogen only formulations and combined formulations, RR 1.30 (95% CI: 1.21 to 1.40), p<0.0001; and RR 2.00 (95% CI: 1.88 to 2.12), p<0.0001 respectively. A similar but stronger association for estrogen only HRT was observed in the Danish Nurses cohort study (2004)[165] (10,874 women), RR 1.96 (95% CI: 1.16 to 3.35) and for E + P combined formulations: RR 2.70 (95% CI: 1.96 to 3.73).

A number of pooled analyses of epidemiological studies on HRT use and breast cancer risk have been conducted and include those by Lee et al. (2005)[166], Shah et al. (2005)[167], the Collaborative Group on Hormonal Factors in Breast Cancer (1997)[158], Steinberg et al. (1994)[168], Silero-Arenas et al. (1992)[169], and Steinberg et al. (1991)[170], of which there is some overlap in studies. A summary of these studies is described in **Table 3.2**. The pooled analyses all suggest that HRT, estrogen alone or combined E + P formulations, increase breast cancer risk.

Two meta-analyses, by Bush et al. (2001)[171] and Greiser et al. (2005)[172], have also been published. The former analyses found little consistency among the observational studies that examined the association between risk of breast cancer and use of HRT (ever versus never) and duration of use. On the other hand, the latter study, which included the larger more recent randomised controlled trials, found a linear increase in overall risk of invasive breast cancer with HRT use. Annual increases in BC risk ranged between 0–9% for estrogen and progestagen combined formulations and 0–3% for estrogen only.

Table 3.2: Pooled analyses of epidemiological studies examining the association between HRT use and breast cancer risk.

Study	Studies (N)	Women (N)	Hormone	Pooled estimates
Lee et al. (2005)[166]	61*	-	E & P	OR 1.08 (95% CI:1.07 to 1.08) per year of use
Shah et al. (2005)[167]	13	700,000	E	OR 1.16 (95% CI: 1.06 to 1.28) current use
	8	650,000	E & P	OR 1.39 (95% CI: 1.12 to 1.72) current use
Collaborative Group on Hormonal Factors in Breast Cancer (1997)[158]	51	209,594	HRT	RR 1.35 (95% CI: 1.21 to 1.49) HRT ≥5 years RR 1.02 (95% CI: 1.01 to 1.04) per year of use
Steinberg et al. (1994)[168]	20	~45,000	E	Δ RR 0.00013 (95% CI: 0.0008 to 0.0018) per month of estrogen use RR 1.15–1.49† for 10 years of use
Silero-Arenas et al. (1992)[169]	27	-	HRT	RR 1.06 (95% CI: 1.00 to 1.12) ever use
	12			RR 1.17 (95% CI: 1.06 to 1.29) 4–8 years of use
Steinberg et al. (1991)[170]	16	-	E	RR 1.3 (95% CI: 1.2 to 1.6) for ≥15 years of use

* Includes 51 studies by Collaborative Group

† Different RRs because used different models of study inclusion

To further support the link between HRT use and breast cancer risk a recent Australian study examined the association between a fall in HRT use (since the reporting of the Women's Health Initiative findings on HRT and breast cancer risk) and breast cancer incidence and found a reduction in breast cancer incidence with reduced HRT use[173]. This was also observed in another recently published study[174].

3.3.3 Oral contraceptive pill

As described above, the contraceptive pill contains ethinyl estradiol, but in much lower doses (30–50 μg/d) than that used in the treatment of tall girls (150 μg/d), though higher doses, in the form of mestranol, were used in earlier years. A large number of studies have examined the association between general OC use and breast cancer, with more than 60 case-control studies and 10 cohort studies, several meta-analyses and a large pooled analysis among them[175]. A pooled-analysis of 34 case-control studies (Kahlenborn et al. 2006)[176] that examined the association between OC use and premenopausal cancer reported an increased risk of breast cancer with women who had ever used OCs (RR 1.19, 95% CI: 1.09 to 1.29) and an increased risk with OC use in parous women before first full-term pregnancy (FFTP) compared with women who used OC after FFTP (RR 1.44, 95% CI: 1.28 to 1.62). This data displayed a larger but similarly positive association between OC use (ever used) and breast cancer risk that was observed in an earlier meta-analysis[177] of 34 studies, and a separate pooled analysis of 54 studies[178]. This earlier pooled analysis observed an increased risk of having had a breast cancer diagnosis: current users RR 1.24 (95% CI: 1.15 to 1.33), 2p<0.00001; 1–4 years after stopping RR 1.16 (95% CI: 1.08 to 1.23), 2p=0.00001; five to nine years after stopping RR 1.07 (95% CI: 1.02 to 1.13). No significant excess risk of having breast cancer diagnosed 10 or more years after stopping use was observed [relative risk 1.01 (95% CI: 0.96 to 1.05)]. Interestingly, this pooled analysis found that women who began use before age 20 had higher relative risks of having had breast cancer diagnosed while they were using combined oral contraceptives or within the five year period after stopping use, when compared with women who began use at older ages. The estimated excess number of cancers diagnosed for women who used OC within the 10 years prior, was found to be 0.5 (95% CI: 0.3 to 0.7) among 10,000 women who used OCs from age 16–19 years.

A subsequent case-control study not included in the meta-analyses above, elucidated the combined effects of OC use and genetic factors in a population-based series of BRCA1/2 mutation-tested early-onset breast cancers and found a significantly increased risk for early-onset breast cancer for each year of OC use prior to age 20 years[179]. This association between

longer durations of use before age 20 and increasing risk of early-onset breast cancer risk was also observed in an age-matched case control study that found for those women diagnosed before 36 years, risk increased for longer OC use before age 20 (RR 1.44 per year, p=0.04)[180]. Confidence intervals were not provided.

In light of the overall evidence available on oral contraceptive use and breast cancer risk, in 2005, The World Health Organization classified combined estrogen and progestagen contraceptives as group 1 carcinogens[181]. In relation to the treatment of tall stature with high-dose estrogens, Drop et al. 2001)[50] (p 981) stated:

> *"In view of a relationship with oral contraceptive (OC) use at an early age and duration of OC use with increased risks of breast cancer... there is a need for long-term follow up of individuals treated with pharmacological doses of estrogens."*

3.3.4 Diethylstilbestrol (DES)

The evidence above suggests that high endogenous levels of estrogen and exogenous estrogen exposures, in the form of hormonal replacement therapy are associated with increased risk of breast cancer risk. Diethylstilbestrol is another form of exogenous estrogen that has also been studied extensively in relation to breast cancer risk. Diethylstilbestrol was used by pregnant women to prevent miscarriage. Typically, the dose of DES was 5 mg a day beginning in the sixth week of pregnancy, increasing to 125–150 mg/d in the 35th week[182]. This form of estrogen was also used in the treatment of tall stature in adolescent girls (typically 3 mg twice a day 4–5 days a month) but ceased to be used because of reports in 1971[58] that it was associated with rare clear cell adenocarcinoma of the vagina in the daughters of women treated with DES in pregnancy to prevent miscarriage. These findings have led to a number of follow-up studies examining the long-term effects of DES treatment on reproductive tissue later in life, in particular breast cancer risk in the daughters who were exposed *in utero*, and the mothers who were exposed while pregnant. A review of these findings is presented below.

3.3.4.1 In utero exposure to DES

A cohort study involving 3,674 DES women exposed *in utero* and 1,219 unexposed women of mean age 38 years (data collection 1994), found no increased risk of breast cancer in DES exposed women[183]. A second follow-up (data collection 2003) of the same cohort (with an additional sample of women) involved 3,812 exposed and 1,637 unexposed women. The investigators found that females exposed to DES *in utero* were 40% more likely to develop breast cancer than unexposed women (age-adjusted IRR 1.40, 95% CI: 0.89 to 2.2) though this finding was not statistically significant[184]. The risk, however, was significantly increased for women aged 40 years and over (IRR 1.91, 95% CI: 1.09 to 3.33) and in women 50 years and over (IRR 3.00, 95% CI: 1.01 to 8.98).

A re-analysis of the above DES Combined Cohort Follow-up Study[185] was undertaken to assess overall cancer risk, in addition to breast cancer, using age and calendar-year specific standardised incidence rate ratios (SIR), and age-adjusted incidence rate ratios (RR). This study identified breast cancer cases in 97,831 and 34,810 person-years for DES exposed and unexposed women, respectively. Breast cancer risk was only elevated in women over 40 years who were exposed *in utero* (RR 1.83, 95% CI: 1.1 to 3.2). While a limitation of this study was the incomplete retrieval of medical records to confirm breast cancer cases, restricting the analysis to pathology confirmed cases did not alter the relative risks[185]. Loss to follow-up was also a concern for this study. However, cancer deaths were followed up through the National Death Index, and cancer risk factors were found not to differ between those who had participated and those who did not[185].

3.3.4.2 Exposure to DES during pregnancy

More studies have investigated the risk of breast cancer in women exposed to DES during pregnancy (i.e. as mothers), compared to the daughters who were exposed prenatally. Women exposed to DES during pregnancy are older than women who were exposed *in utero,* providing a more optimal opportunity for breast cancer outcomes to be observed.

Three randomised controlled trials (Bibbo et al. 1978[186], Vessey et al. 1983[187], and Beral et al. 1980[188]) originally designed to examine treatment effectiveness of DES during pregnancy were later followed-up 20–27 years post-pregnancy. No association was observed between treatment with DES and breast cancer, however, these three studies had small sample sizes (n=693, 319 and 79 treated women, respectively) and few cases of breast cancer.

Later studies were more robust in their design and had larger sample sizes. Hadjimichael et al. (1984)[189] investigated breast cancer risk with larger doses of DES exposure (mean 2 g) in a large cohort of 1,707 US women exposed to DES during pregnancy, and 1,405 unexposed women matched for age, race, sex of offspring and date of pregnancy. This retrospective cohort study followed up 68% of DES exposed and 70% of unexposed women and identified breast cancer cases via cancer registry data. The adjusted relative risk for breast cancer in the treated group compared to the untreated group was 1.37 (95% CI: 0.83 to 2.28).

The DES Mothers Study[190], published the same year as Hadjimichael above involved a cohort of 3,033 mothers exposed to DES in pregnancy through 1940–1960 (dose not reported), and an age-matched comparison group of similar size. Women exposed to DES were found to have a 40% greater risk of developing breast cancer than untreated women (Crude Relative Risk 1.4, 95% CI: 1.1 to 1.9). A similar result was observed with two sequential follow-ups of this study cohort. In the second follow-up (Colton et al.1993)[191] 93% of exposed and 89% of unexposed mothers from the original DES Mothers Study participated. A relative risk of 1.35, (95% CI: 1.05 to 1.74) was observed. The third follow-up (Titus-Ernstoff et al. 2001)[192] involved a combined analyses of two cohorts, one of which was the DES Mother's Study. This study demonstrated a modest increased risk of breast cancer in women treated with DES in pregnancy RR 1.27 (CI: 1.07 to 1.52), a finding that is consistent with the earlier studies. These similar results over different lengths of follow-up suggest that the risk did not increase over time as the cohort aged.

An increased risk of dying from breast cancer has been associated with DES use in pregnancy. A retrospective cohort study by Calle et al. (1996)[182] observed 1,574 cases of fatal breast cancer among 501,536 gravid women who reported no prior history of cancer. Results showed a positive association between a history of DES exposure during pregnancy (reported by 3.9% of all women) and fatal breast cancer (adjusted rate ratio 1.34, 95% CI: 1.06 to 1.69). This excess risk did not increase over time; women who were exposed more than 35 years ago (rate ratio 1.35, 95% CI: 0.97 to 1.87) were not at greater risk than women who were exposed within the past 35 years (rate ratio 1.39, 95% confidence interval 1.01 to 1.93). The positive association was not observed in women who used DES before age 25 years but was seen at all other ages [rate ratio 1.00 (95% CI: 0.59 to 1.71)].

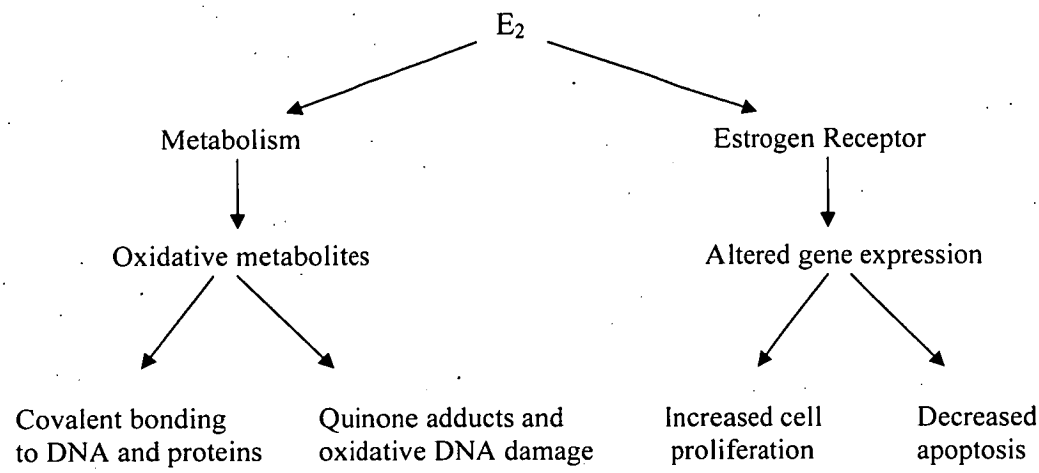
The available evidence supports a modest increase in breast cancer risk in women exposed to DES while pregnant and at a time of intense mammary gland proliferation. Guisti and colleagues in their 1995 review[193] of DES in pregnancy and breast cancer risk concluded a less than twofold increase in breast cancer risk.

In summary, it appears that DES during pregnancy, and possibly DES exposure *in utero* (for ages 40 years and older) are associated with an increased risk of breast cancer later in life. Studies on the latter group need to wait longer for these women to be at an age when breast cancer incidence is at its greatest.

3.3.4.3 Mechanism of estrogen carcinogenicity

Yager and Davidson recently reviewed the findings related to the risk of breast cancer with estrogen exposure and the mechanisms that may be involved[194]. The potential mechanisms fall within two broad actions, the genotoxicity of estrogen metabolites and the stimulation of tissue proliferation. See **Figure 3.3**.

Figure 3.3: Pathways for estrogen carcinogenesis.



Modified Figure 1 of Yager & Davidson (2006)[194]

As well as these two potential mechanisms of carcinogenesis, it is possible that estrogen acts through other mediators such as prolactin or IGF-I. Prolactin and IGF-I serum levels have been associated with increased breast cancer risk and have been observed to change in girls treated with high-dose estrogens for tall stature. A recent review[195] of epidemiological, animal and molecular biology findings by Harvey and colleagues strongly supports the postulate that prolactin is a risk factor for human breast cancer[195]. This view is

further supported by additional later studies[196, 197]. Similarly, a large number of epidemiological studies have examined the association between plasma IGF-I levels and breast cancer risk. Hankinson and Schernhammer[198] in their 2003 review of epidemiological studies of association between circulating IGF-I and breast cancer risk observed an overall positive association between the two, depending on menopausal status. In their review, the prospective studies tended to demonstrate a strong positive association in premenopausal women, but no association among postmenopausal women. This has been supported by a subsequent case-control study[199] nested within the Nurses Health Study cohort (n=800 cases, and 1,129 aged-matched controls) but is not supported by a smaller study[200] (n=317 cases, 634 age-matched controls) nested within the Nurses Health Study II cohort.

While evidence supports an association in menopausal women only, a case-control study[201] found an association with estrogen receptor positive breast cancers only: progesterone receptor positive (ER+PR+) breast cancer (OR 2.4, 95% CI: 1.1 to 5.4) and ER+ve PR--ve breast cancer (OR 4.3, 95% CI: 1.2 to 14.3).

Of particular interest is the study by Lukanova et al. 2006[202] that found a stronger association between IGF-I levels and breast cancer risk among primiparous (OR 2.2, 95% CI: 1.1 to 4.4) compared with the non-primiparous (OR 1.4, 95% CI: 0.7 to 2.8), suggesting a stronger influence of IGF-I on the breast before the remodeling of the gland induced by a first pregnancy. Upper-tertile risks decreased with age: from OR 2.5 (95% CI: 0.9 to 7.6) to OR 2.1 (95% CI: 0.9 to 5.0) and OR 1.2 (95% CI: 0.5 to 2.5) for the age groups <28, 28 to 33, and >33 years of age, respectively.

3.3.5 Adolescent hormonal exposures

The adolescent developing breast is undergoing one of three periods of increased mammary tissue proliferation (*in utero*, pubertal development, and pregnancy). The rudimentary mammary structures that follow the neonatal period are quiescent throughout childhood[9], awaiting stimulation by the hormonally regulated pubertal stage of breast development. According to De Silva and Brandt[203], the pubertal stage of breast development, that occurs over a period of two to four years, involves the growth of adipose tissue and lactiferous ducts in response to estrogen. This is followed later (when the corpus luteum matures) by progesterone stimulated tertiary branching and alveolar-lobular budding at the ends of these ducts[89, 203, 204]. Consequently, this period of mammary gland development may be sensitive to hormonal exposures and therefore susceptible to breast disease initiation or development.

It has been proposed that the three stages of increased mammary gland proliferation: *in utero*, puberty and pregnancy, have an increased susceptibility to mammary gland carcinogenesis[205, 206], with recent interest expressed towards hormonal exposures during adolescence and breast cancer risk[30, 207]. It has been postulated that breast tissue during the adolescent growth period is more susceptible to carcinogenesis than later periods in life[207] and that research efforts should place greater attention on this life-stage in particular. As mammary cellular proliferation is stimulated by exposure to estrogen and progesterone[32, 33], it is possible that the degree of exposure to these hormones during pubertal mammary development when glandular tissue may be particularly sensitive to carcinogenic insults[208], may influence the risk of breast cancer in later life.

In concordance with this, it has been suggested that lower levels of these sex hormones during adolescence could potentially protect against breast cancer by altering breast morphology through a reduction in the rate of cell turnover and proliferation[34]. Early age of menarche is a well established breast cancer risk factor[35], and it is suggested that this may be attributable to longer or earlier lifetime exposure to estrogen and progesterone, especially during the critical period of breast development.

Further evidence that the mammary gland may have a heightened sensitivity during the adolescent period comes from findings in a rat model of carcinogenesis where cancer initiation required the integration of chemical carcinogens with undifferentiated and highly proliferating mammary epithelium[31]. The cells of the pubertal mammary gland are highly proliferative and undifferentiated. Differentiation of the mammary gland, such as that induced by full-term pregnancy, was found to inhibit carcinogenic initiation. This is supported by studies linking breast cancer later in life to exposures to carcinogens or estrogenic pollutants (e.g. DDT) during adolescence[209, 210].

Girls who are treated with high-dose estrogens for tall stature also took a progestagen four to five days a month to promote cyclic bleeding. These girls were prematurely exposed to a progestagen. Normal ovulatory cycles do not start until late puberty. Girls experience one to two years of anovulatory menstrual periods involving estrogen stimulation of the breast epithelium. When ovulatory cycles begin the corpus luteum matures and secretes progesterone. It is uncertain to what degree this premature exposure to a progestagen has on breast development.

According to Vorherr[9] estrogens are responsible for the first major increase in mammary tissue in preadolescent females which is followed later, at onset of ovulation, by the combined influence of estrogen and progesterone on the breast. Progesterone is involved in lateral branching[204] and alveolar lobular development at the terminal end buds[203]. The effect of premature exposure to progesterone on the degree of ductal development and/or lobular growth, and the structure or density of the terminal ductal lobular units is unknown. In the adult human breast, the highest proliferative activity is observed to occur during the luteal phase of the menstrual cycle, a time when levels of both estradiol (E2) and P are high[211]. In addition, estrogen and progesterone hormone replacement therapies (HRT) used in menopausal/postmenopausal women have been associated with an increased breast cancer risk compared with HRT formulas containing only estrogen[167]. This may be due to the added proliferative activity of the combined therapy. It is possible that the combined estrogen and progestagen treatment for tall stature increases the proliferative activity of the breast and hence the risk of breast cancer later in life.

3.4 Lactation

When the evidence above is considered together with the reported changes in the hormonal milieu of girls treated with estrogens as described in Chapter 2 and previous findings from the Australian Tall Girls Study on the long-term effects of treatment on fertility, it seems possible that treatment could have long-term effects on breast histology and therefore, function. This section explores the few available epidemiological and animal studies that have investigated the effect of exogenous estrogen exposures on lactation[†††]. These studies suggest long-term effects of pubertal estrogen exposures on nipple structure and lactation ability. It is also evident, from the review of studies, that no other study has examined the long-term effect of high-dose estrogen treatment in adolescent girls on mammary function later in life.

3.4.1 Effect of exogenous estrogen exposures on lactation

Very few epidemiological studies have examined pubertal or adolescent hormonal exposures on lactation. A number of epidemiological and animal studies have examined the effect of population level exposures to estrogenic pesticide pollutants such as 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT), or its metabolite, 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE) on lactation. An overview of these studies is presented below and summarised in **Table 3.3**.

[†††] Studies were identified by PubMed search of the English language literature using the terms lactation (OR breastmilk OR breast-milk OR 'milk AND production/initiation/duration/quality) AND hormone OR o/estrogen OR DES/DDE/DDT OR contraceptive OR progestagen/progesterone) for any field covering all dates up to the time of writing. The reference lists of all the publications identified by this search were inspected for additional studies. The findings and characteristics of all animal and human studies and/or reviews that explored and reported the association between estrogen exposure and lactation were described.

3.4.1.1 The effect of DDT/DDE exposures on lactation initiation and duration

Rogan et al. (1987)[212] investigated the effect of exposure to the pesticide metabolite DDE on breast feeding duration in mothers who had not previously breastfed. They followed up 802 babies from birth to one year of age in North Carolina USA, and observed a reduced duration of breastfeeding in the children of mothers with higher concentrations of DDE in their breast milk compared to children of mothers with lower levels: age and socio-economic adjusted regression coefficient –0.9 (95% CI: –1.7 to –0.1). They also observed a positive association between DDE levels and rates of lactational failure.

Similar findings were observed in another study by the same authors, published eight years later[213]. This cross-sectional study of 229 Mexican women, observed median breastfeeding duration of 7.5 months in women with the lowest DDE levels in their breastmilk, compared to three months in those with the highest levels. The effect, however, was only observed in women who had lactated previously.

Table 3.3: Summary of studies that have investigated the association between environmental estrogen exposures as measured by serum or breast milk concentrations and breastfeeding duration or initiation.

Study	N	Estrogen	Source	Outcome	Finding	Adjusted
Rogan et al. (1987)[212]	802	DDE	Breastmilk	Duration of breastfeeding	Regression Coefficient −0.9 (95% CI: −1.7 to −0.1)	Age and SES
Gladen & Rogan (1995)[213]	229	DDE	Breastmilk	Duration of breastfeeding	HR 0.7 (95% CI: 0.4 to 1.5) for 2.5–5.0 versus 0–2.5 ppm HR 1.3 (95% CI: 0.6 to 2.6) for 5.0–7.5 ppm HR 1.3 (95% CI: 0.6 to 2.5) for 7.5–10.0 ppm HR 1.3 (95% CI: 0.6 to 3.0) for 10.0–12.5 ppm HR 2.6 (95% CI: 1.1 to 5.9) for >12.5 ppm Only in women who previously breastfed	Multi-variable
Karmaus et al. (2005)[214]	91	DDE	Serum	Breastfeeding initiation	IR 0.42 (95% CI: 0.10 to 1.03) for ≥10 mg/L versus 0<5 mg/L only in women who had previously attempted to initiate breastfeeding	Birth cohort, maternal age at birth, education of the mother, and smoking during pregnancy
				Duration of breastfeeding	Inverse association between DDE concentration and duration HR: 8.73 (95% CI: 1.84 to 41.5) in women who had not previously breastfed	

Study	N	Estrogen	Source	Outcome	Finding	Adjusted
Weldon et al. (2006)[215]	366	DDT DDE PCB	Serum	Duration of breastfeeding	No association with DDT or DDE PCB (HR 1.5, p=0.02)	Parity, years in US, poverty status, marital status, caesarean delivery, maternal age, education, occupation, and BMI
Cupul-Uicab et al. (2008)[216]	784	DDE	Serum	Duration of breastfeeding Breastfeeding initiation	HR 1.40 (95% CI: 1.06 to 1.87) for 3–6 µg versus ≤3 µg only in women who had previously breastfed. OR 1.96 (95% CI: 1.18 to 3.26) in women who had not breastfed previously	-

DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane
DDE: 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene
PCB: polychlorinated biphenyl
ppm=parts per million

Karmaus et al. (2005)[214] (**Table 3.3**) examined both initiation and breastfeeding duration with maternal serum DDE concentrations in non-smoking women and observed an incidence ratio for breastfeeding initiation 0.42 (95% CI: 0.10 to 1.03) when maternal serum DDE concentrations were ≥10 mg/L, compared with the lowest DDE exposure group. However, no effect was observed when the analysis was restricted to women who had never previously attempted to initiate breastfeeding. In the offspring (of non-smoking mothers), breastfeeding duration was shorter when DDE concentrations were higher: 13 weeks for ≥10 mg/L DDE, compared with 30.3 weeks for lower DDE in women who had not previously breastfed (adjusted HR: 8.73, 95% CI: 1.84 to 41.5)[214]. The sample size was small in each group e.g. n=9 for ≥10 mg/L DDE and 19 in the reference group.

Weldon et al. (2006)[215] (**Table 3.3**) found no association between DDE or DDT and breastfeeding duration in a cohort of 366 US women but did find an association with polychlorinated biphenyl (PCB), which has estrogenic properties (See **Table 3.3**).

Cupul-Uicab et al. (2008)[216] (**Table 3.3**) observed similar results to Gladen and Rogan (1995)[213] in relation to breastfeeding duration. In their cross-sectional study of 784 Mexican women, they found an association between DDE serum levels and breastfeeding duration in women who had previously breastfed, but they did not observe an association with women who had not previously breastfed. In women who had previously breastfed, the adjusted hazard ratios of weaning for DDE concentrations 3.01–6.00 μ/g; 6.01–9.00 μ/g; and >9.00 μ/g, compared to DDE concentrations ≤3.00 μ/g lipids, were 1.40 (95% CI: 1.06 to 1.87); 1.91 (95% CI: 1.24 to 2.93); and 1.76 (95% CI: 1.22 to 2.53) respectively.

Women who had breastfed previously have reduced levels of DDT stored, and hence metabolised (to DDE) and available in serum, compared with women who had not previously breastfed[213]. Cupul-Uicab et al. (2008)[216] argued that the effect should have been observed in both groups of women, those who had previously breastfed and those who had not, and suggested that the effect observed in women who had previously breastfed must be due to a non-causal association. According to Cupul-Uicab et al. (2008)[216] and Gladen and Rogan (1995)[213], a woman who breastfed longer in her first lactation will tend to breastfeed longer the second time as well, and, because DDE is excreted in milk, women with longer periods of lactation would eliminate

more DDE than women with shorter durations, creating a non-causal association between higher DDE and shorter periods of lactation in women who previously breastfed[216].

Like Karmaus above, Cupul-Uicab et al. (2008)[216] examined breastfeeding initiation. The adjusted odds ratio for having had problems with breastfeeding initiation per unit increase in DDE levels (natural log scale) for women who had not breastfed previously was 1.96 (95% CI: 1.18 to 3.26) and for women who had previously breastfed: OR 0.97 (95% CI: 0.60 to 1.57)[216]. The study investigators suggested that women who had not breastfed previously may be more susceptible to endocrine effects on lactation initiation.

Cupul-Uicab[216] and colleagues have suggested that DDE and therefore DDT, and possibly other estrogenic compounds, only affect the establishment of lactation, not duration. Milk production is only under endocrine control for the first two to three days after which, if lactation has been established, autocrine mechanisms mainly operate[217]. Cupul-Uicab et al. (2008)[216] surmised that DDE might reduce milk production during these early endocrine controlled days, but if lactation still managed to be established, and autocrine control of lactation dominated, DDE would no longer have any effect on lactation. A 1977 BMJ article (anonymous author)[218] reported that estrogens are more effective at preventing initial milk production, than stopping lactation once established. If this is true, it is unclear why Karmaus et al. (2005)[214], observed shorter breastfeeding durations at higher DDE concentrations in women who had not previously breastfed[214].

Estrogenic pesticides such as DDT and its metabolite DDE are stored in fatty deposits including the fatty component of breast milk and are released into the serum. It is impossible to separate these effects on lactation and attribute them to any particular period of exposure – it may represent cumulative exposure over a lifetime or it may represent current exposure.

Epidemiological studies that have examined estrogen exposures during adolescence and subsequent lactation performance have not been found in the literature. One possible area of investigation is the association between oral contraceptive use at a young age and subsequent effects on lactation. A number of studies have examined the effect of oral contraceptive use on lactation, but these exposures either occurred after pregnancy, during lactation[219-221], or shortly

before it[222]. A number of animal studies have examined the effect of prepubertal or pubertal exposures to estrogens on lactation. A review of these studies is presented below.

3.4.1.2 Animal studies of early-life hormonal exposures and lactation

Animal studies have investigated the effect of early-life hormonal exposures and lactation ability. The effects of prenatal and prepubertal/pubertal exposures are described separately below.

Prenatal exposures

Prenatal exposures to estrogens have been reported to alter mammary gland development in rats[223-227] that has persisted into adulthood (See **Table 3.4**). Whether these morphological effects translated to lactational effects was not explored. One study indirectly examined the effect of prenatal exposures to estrogenic substances on later lactation[226]. Rayner et al. (2005)[226] exposed rats prenatally to atrazine, a herbicide with estrogenic properties. The female rats displayed delayed mammary gland development when examined in adulthood. Compared to control rats, the glands of treated animals had less epithelial branching, and contained many more terminal end buds suggesting lack of maturity of the gland. The offspring of these exposed rats had a lower mean body weight than the offspring of control rats[226]. The researchers speculated that this was due to poor lactational support of the offspring suggesting that *in utero* exposure to estrogens can have long-term effects on mammary gland development and lactation as an adult.

Table 3.4: Summary of animal studies on the effect of *in-utero* and prepubertal estrogen exposures on the mammary gland.

Study	Animal Model	Estrogen	Dose	Stage at Exposure	Outcome
Markey et al. (2001)[227]	Rat	Bisphol-A	25 and 250 µg/kg/day	*in-utero*	Increased percentage of ducts, terminal ducts, terminal end buds, and alveolar buds
Fenton et al. (2002)[225]	Rat	Dioxin	1 µg TCDD/kg/day	*in-utero*	Reduced primary branches, decreased epithelial elongation, and significantly fewer alveolar buds and lateral branches
Foster et al. (2004)[224]	Rat	Genestein	10 µg/g/day	*in-utero*	Ductal hyperplasia and fibrosis
Rayner et al. (2005)[226]	Rat	Atrazine	100 mg/kg/day	*in-utero*	Delayed mammary gland development and poor lactational nutritional support of their subsequent offspring
Fielden et al. (2002)[223]	Mice	DES	0, 0.1, 1, 10 µg/kg/day	*in-utero* and lactation	Increase in mammary gland growth Decrease in terminal end buds
Golub et al. (2003)[26]	Rhesus Monkeys	Methoxychlor (MXC)	25 and 50 mg/kg/day	peripubertal	Retarded growth of the nipple for both estrogens
		DES	0.5 mg/kg/day.		
Lammers et al. (1999)[27]	Heifers	Estradiol-17b	NS	prepubertal	Reduced teat length by 30% First lactation milk production decreased by 5.2%
Folley (1956)[28]	Goats	Hexoestrol	0.5 mg/day	prepubertal	Lobular alveolar growth but abnormalities e.g. large or cystic alveoli, immature lobules, papillomatous outgrowths and an overall deficiency of secretory epithelial surface area[28]

NS: Not stated

Postnatal exposures

Whether pubertal exposures exert long-term effects on later lactation is unknown. Animal studies have suggested that mammary development in the pubertal period is critical for future lactation[228]. Studies involving rhesus monkeys[229], prepubertal heifers[27] and goats[28] have reported effects of estrogen on ductal and lobular/alveolar proliferation, nipple structure and lactation (see **Table 3.4**)

Folley (1956)[28] exposed young ovariectomised virgin goatlings with the estrogen hexoestrol. They compared the mammary tissue of the subsequent lactating animal when compared with the mammary tissue of unexposed lactating goats. They observed excessively large or cystic alveoli, immature lobules, papillomatous outgrowths and an overall deficiency of secretory epithelial surface area[28]. Lactation performance was not measured, but it is possible that these abnormalities lead to deficiencies in lactation. The addition of a progestagen reduced the incidence of abnormalities.

Golub et al. (2003)[26] examined the consequences of treating female rhesus monkeys with high-dose estrogen (n=8 per treatment type) during the peripubertal period (six months before and following the expected age at menarche). The estrogens used included the estrogenic pesticide methoxychlor, (MXC), 25 and 50 mg/kg/day; and diethylstilbestrol (DES), 0.5 mg/kg/day. DES completely suppressed adolescent growth (weight and height) while both estrogens led to swelling of skin, and retarded growth of the nipple compared to control animals (n=8)[26]. While effect on lactation was not examined, a consequence of retarded nipple growth might be reduced ability to initiate lactation.

Lammers et al. (1999)[27] observed a similar effect of high-dose prepubertal estrogen exposure on teat growth in heifers and also explored the effect of exposure on lactation. Fourteen prepubertal heifers were exposed to estrogen implants over a 20 week period. Estrogen implants initially stimulated a large increase in teat length growth during the treatment period, but the advantage was lost post-treatment. Over the treatment and post-treatment periods the estrogen implants reduced teat length by 30%. The effect of the implants on lactation milk volume was also examined. First lactation milk production decreased by 5.2%[27].

3.4.2 Estrogen exposure in adolescence: possible effects on lactation

For the first three to four weeks of pregnancy the mammary gland, in preparation for future lactation, undergoes ductular sprouting and branching, and lobular formation[9]. At about five to eight weeks into pregnancy, women should notice a definite enlargement and feeling of heaviness of the breasts[9]. The extent of this mammary change during pregnancy depends on the woman's pre-pregnancy size, the number of mammary lobuli, and the age and parity of the woman[9]. Since pre-pregnancy size and number of mammary lobuli may be altered by high-dose estrogen exposure during adolescence, then mammary change during pregnancy and subsequent lactation may also be affected.

Breast hypoplasia or insufficient glandular development could result in lactation failure. An associated characteristic of women with breast hypoplasia is absence of typical breast changes with pregnancy and failure of postpartum breast engorgement leading to a failure or reduced ability to lactate[230]. These changes that are expected to occur during pregnancy may depend on events in adolescence. Anecdotal reports of incomplete breast development or 'flat chestedness' as a side effect of treatment for tall stature has been published elsewhere[23, 41].

According to Tucker (2000)[231], in his historical perspective of mammary growth and lactation; hormones are involved and absolutely necessary for the initiation of lactation, but not without a well developed mammary lobule-alveolar system. It is possible that the hormonal and physiological effects observed during treatment for tall stature have longer term effects on the mammary lobule-alveolar system and subsequently, later lactation.

The attenuation of IGF-I levels in girls treated with high-dose estrogens for tall stature (See Chapter 2, Section 2.4.2.2) could lead to changes to mammary development and functional deficiencies. Two studies, one by Lammers et al. (1999)[27] and the other by McCann et al. (1989)[232] found rapid weight gain at puberty to impair mammary gland development and later milk production in the heifer[27] and ewe[232]. One possible reason for the effect on mammary development and later milk production in the animal model, is a reduced sensitivity of the tissues to IGF-I[27]. Weight gain and reduced IGF-I levels have been reported to occur in adolescent girls treated with high-dose estrogens (Chapter 2, Sections 2.4.3.1 and

2.4.2.2 respectively), further supporting the suggestion that treatment could affect mammary gland development and subsequent lactation

3.5 Overview

The above review examined Australian and international published case-series reports of adverse effects on the breast during and shortly following cessation of estrogen treatment in tall girls. The review highlighted the limitation of existing reports on the short-term side effects of treatment on the breast. The prevalence of effects by treatment type has not been reported, nor have all side effects been examined consistently across studies. Most of the case-series reports were based on small sample sizes, and the outcomes were selected by the treating physicians rather than independent researchers. For uncommon outcomes, it is unclear the degree to which physicians systematically examined girls for these conditions in the studies, and subsequently reported them. There is a need for a more systematic examination of side effects of treatment on the breast in relation to treatment type.

The review also highlighted the gaps in our understanding of the long-term effects on the breast, brought about by the scarcity of long-term follow-up studies, and the importance of this PhD research in narrowing this gap. Published epidemiological, molecular, *in vitro* and *in vivo* studies examining the association between estrogen and benign breast disease and breast cancer risk were presented. The evidence suggests that estrogen (and progestagen) exposure in adolescence could influence the risk of these breast diseases later in life.

The review above also described findings from epidemiological and animal studies on the effect of estrogen exposures on nipple structure and lactation. While caution is required when extrapolating effects on animal tissues and physiological functions to humans; when combined with evidence of breast related side effects described above, it is plausible that high-dose estrogen exposure during puberty, at a time of intense mammary development, could have long-term effects on lactation. No other study has examined the long-term effect of this treatment on mammary function.

Box 3.1: Key points from the literature in Chapter 3.

KEY POINTS FROM THE LITERATURE: CHAPTER 3

- Reported breast related short-term side effects of treatment with high-dose estrogens in adolescent girls include breast pain, pigmentation of the areolae and nipples, galactorrhea and, more rarely, benign breast disease.
- Overall, evidence suggests that women who currently use and have used HRT for at least five years are at increased risk of benign breast disease and breast cancer.
- DES exposure in pregnancy appears to be associated with an increased risk of breast cancer later in life.
- Few studies have examined the effect of hormone exposures in adolescence on later lactation in humans. However, studies that have examined the association between environmental estrogen exposures (DDT, DDE) on lactation in humans suggest that exposure to high-doses of these estrogenic chemicals can affect the initiation of lactation.
- High-dose estrogen exposure at a time of intense mammary development has been shown to affect nipple/teat length and structure, epithelial growth, and lactation volume in the animal model.
- Breast hypoplasia, or insufficient glandular development, could result in lactation failure. An associated characteristic of women with breast hypoplasia is absence of typical breast changes with pregnancy and failure of postpartum breast engorgement leading to a failure or reduced ability to lactate.
- Studies have found rapid weight gain at puberty to impair mammary gland development and later milk production in the animal model. Weight gain and reduced IGF-I levels have been reported to occur in adolescent girls treated with high-dose estrogens.

4: TREATMENT WITH HIGH-DOSE ESTROGENS IN ADOLESCENCE: SHORT-TERM EFFECTS ON THE BREAST AND LONGER TERM BREAST DISEASE

4.0 Introduction

Chapter 3 highlighted the gaps in our understanding of the short- and long-term effects on the breast of high-dose estrogen treatment in adolescent girls and the importance of this PhD research in narrowing this gap. The Australian Tall Girls Study provided a unique opportunity to examine the prevalence and risk of breast symptoms and disease in women exposed to high-dose estrogen during adolescence.

The Tall Girls Study is a retrospective cohort study. It followed up women who were assessed for tall stature in adolescence and either treated (mean age 39.8 years) or untreated (37.7 years) with high-dose estrogens to reduce their final height. At follow-up 1 of this study, data on the side effects of treatment and breast disease history were collected from both treated and untreated women. This data were available for analysis. This chapter presents the methods and findings of this analysis; in particular, the prevalence of short-term breast related side effects in treated women, and the long-term risk of ever having had a breast biopsy, breast surgery, and breast cancer in treated women compared with untreated women. The cohort size was too small, and participants relatively too young, to expect to pick up many cases of breast cancer. As such, it was not suitable for a reliable assessment of the level of breast cancer risk associated with high-dose estrogen treatment in tall girls (see footnote[‡‡‡]), but it could provide some indication of the incidence in the two groups of women, and whether the rates for treated women appeared out of the ordinary.

‡‡‡ A sample size of approximately 27,000 would be required for each of the treated and untreated groups to detect a 20% relative increase (RR 1.2) in risk for breast cancer in the treated group compared with the untreated group, with 80% power; or 1550 each for a RR of 2.0 (See Appendix 1 for further details of this analysis).

4.1 Study Aim

This study aimed to use data collected in first follow-up of the Australian Tall Girls cohort study to: 1) examine the prevalence of breast related side effects on treated women according to treatment type (e.g DES and EE), 2) test the hypothesis that treated women are at greater risk of developing benign breast disease than untreated women, and 3) to describe the occurrence of cancers in treated and untreated women.

The next section of this chapter presents the methodology used to collect and analyse the data; the findings of this analysis; and finally, the discussion and conclusion.

4.2 Methods

4.2.1 Eligibility

Women were eligible to participate in the Australian Tall Girls Study if they obtained a medical opinion about their tall stature and had a radiological assessment of their skeletal age during adolescence. They included women who received daily estrogen treatment during adolescence, 3 mg diethylstilbestrol (DES) or 150 μg ethinyl estradiol (EE), to reduce their adult height (treated group) and women who had not (untreated). The most common reasons for not being treated with estrogen included: the woman's predicted height as a girl did not warrant treatment, the family preferred not to have treatment or there was little remaining growth suppression potential at the time of the assessment[25].

4.2.2 Recruitment

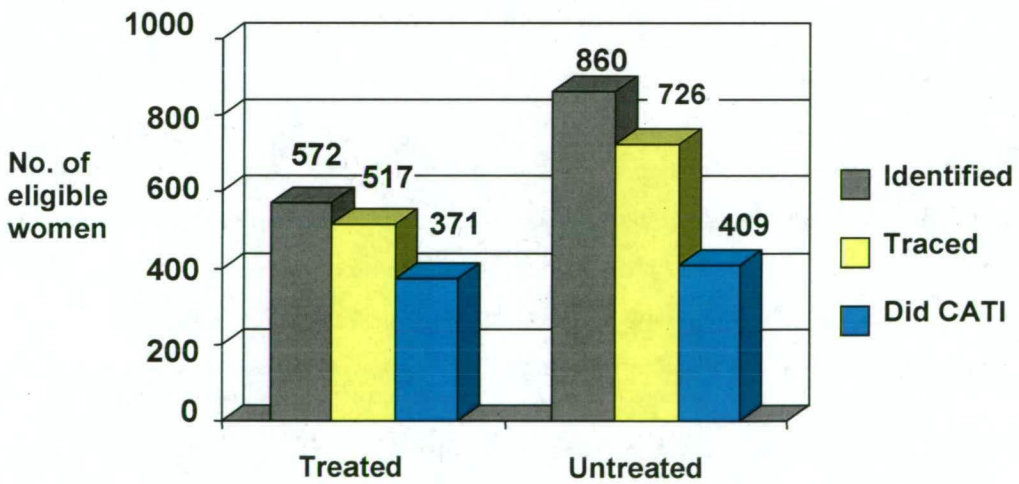
As described elsewhere[25], individuals were identified from medical records of Australian paediatricians who assessed or treated tall girls from 1959 to 1993, and from self-referrals. Women who self-referred to the study included women who had heard about the study through publicity, and members of Tall Girls Inc., an advocacy group for women treated with high-dose estrogens in adolescence. The Australian Paediatic Endocrinology Group (APEG) and specific doctors identified through professional networks were contacted to seek assistance from endocrinologists who had treated tall girls or knew of people who had.

The Tall Girls Study identified a cohort of 1,432 eligible participants: 1,248 from medical records of whom 1,222 were from one paediatric endocrinologist, and 184 from self-referrals. Of this cohort, 572 women were treated and 860 untreated. Women were traced with the use of electoral rolls and telephone listings then a letter of invitation, information brochure, and consent form to participate in the study were sent to the women by mail. The number of eligible women traced and invited to participate in the study was 1,243. Non-respondents were followed up by mail and telephone.

Written informed consent was obtained from all study participants. The study had the approval of the La Trobe University Human Ethics Committee (00/03). See Appendix 2 for a copy of the invitation letter and study information sent to eligible participants.

Of the women invited, 398 treated (77%) of treated and 448 (62%) of untreated women agreed to participate and completed the postal questionnaire. Of these, 371 treated women (72% of traced), and 409 (56%) untreated women completed the computer assisted telephone interview (See **Figure 4.1**).

Figure 4.1: Number of treated and untreated women in the Tall Girls Study who were identified, traced and who participated in the computer assisted telephone interview (CATI).



4.2.3 Exposure data

Treatment information was extracted from the medical records of women who provided consent and for whom records were available, or was self-reported by women in the postal questionnaire used in the first follow-up. Written consent to extract data from medical records was provided by 726 (91% treated, 95% untreated) of the women who completed the

interview, however, medical records were available for only 618 (75% treated, 95% untreated). More records were available for untreated women because a greater proportion of them (97%) compared with treated women (68%) were sourced from one endocrinologist who retained and allowed access to the medical records. More treated women self-referred to the study and had been treated by other endocrinologists whose records could not be accessed.

Type of treatment, and start and end date of treatment (from which duration of treatment was calculated) was collected from the records. If records were not available, women were asked in the postal questionnaire to give the name of the drug that they took (1=DES, 2=EE, 3=other, 4=not sure/can't remember name) and how old they were when they started and completed treatment (years and months) (Questions C22-24 in the postal questionnaire, Appendix 3). They were also asked whether or not they took the tablets regularly (Q C25).

4.2.4 Breast disease

In the postal questionnaire, treated women were asked if they experienced particular breast related side effects while on treatment. These included: increased pigmentation of the nipples, galactorrhea, breast pain, breast lumps, and "other- please specify". They were also asked, "After treatment had finished, did you ever notice a spontaneous leakage of the milk or discharge from your breasts?" (Questions C26-C27 of postal questionnaire in Appendix 3).

During the computer assisted telephone interview women were asked whether they had cancer of the reproductive organs or any other cancer and if yes, what kind of cancer. Breast cancer reports were validated by medical records where possible. In the CATI, women were asked: 'Have you ever had a breast biopsy [sample of breast tissue], a mastectomy [surgery to remove breast], and other breast surgery followed by 'please specify' if yes to the latter (Question A2 in the CATI questionnaire, Appendix 4).

4.2.5 Other covariates

Demographic data including marital status, educational level, and smoking history were collected in the postal questionnaire. The smoking history questions were derived from the Australian Longitudinal Study on Women's Health (Women's Health Australia[233]).
Data on oral contraceptive and fertility drug use, and the use of hormones for a range of reproductive conditions were collected in the telephone interview. Reproductive conditions specified included: cramps or backache associated with menstruation, irregular menstrual cycles, premenstrual syndrome, heavy or prolonged menstrual bleeding; absence of a period for at least six weeks, not due to pregnancy, breastfeeding or taking the pill; menopause, or other menstrual problems.

Data on estimated mature height was collected from the medical records and final height was collected by postal questionnaire at follow-up. Participants were asked to measure their height before noon and in bare feet, standing on a hard floor without carpet. They were asked to stand straight, stretch with their backs against a wall, place a picture frame or similar firm square or rectangular object on their head, and draw a mark on the wall immediately below the frame edge. They were requested to measure the height from the floor to the mark with a tape measure and repeat the process as a check.

Information about mammographic screening history was gathered from the postal questionnaire. The questions were derived from the National Health Survey (NHS). Women were asked if they 'ever' had a mammogram, and if yes, the reason for having their last one. Australian Bureau of Statistics 2001 NHS data on mammographic screening rates were compared with rates for treated and untreated women across age categories. This data provided insight into the potential differences in health screening behaviours and the potential for a disease outcome to be identified or surgical procedure to be performed in both groups.

4.2.6 Data analysis

Statistical analysis was performed with Stata Version 8. Log binomial regression was used to calculate the age-adjusted relative risk of treated women having ever had a breast biopsy, non-elective and elective breast surgery, and breast cancer. Chi-square tests were performed for tests of significance for categorical data, and t-tests for continuous data. All tests of significance were two-sided.

The precise p-values were reported for descriptive data comparisons. Following convention, nominal statistical significance was based on a p-value less than 0.05. Ninety-five percent confidence intervals were described for all log binomial regression generated relative risks (RR). While it is recognised that the 0.05 threshold for p-values and the 95% confidence level is arbitrarily derived (a z value of 1.96 corresponds to a p-value of 0.05), the range of the confidence interval as well as the position of the null value in relation to the interval will be considered when interpreting confidence interval data[234].

4.3 Results

4.3.1 Characteristics of study participants

The characteristics of study participants are shown in **Tables 4.1**. The mean age of participants was 39.8 years (range 20–55) in the treated group and 37.7 years (23–54) in the untreated group. This difference was statistically significant ($p<0.001$). Treated and untreated study participants were similar in their marital status, though slightly more untreated women were single than treated women. Women were similar in the highest educational level achieved in most categories, though more treated than untreated women did not complete secondary school and fewer had a degree. Treated and untreated women were also similar in family income, smoking history, oral contraceptive use and mean age at first livebirth.

Table 4.1: Characteristics of treated and untreated participants.

Characteristic	Treated (N=371)	Untreated (N=409)	P-value
Age			
Mean (years) (range)	39.8 (20–55)	37.7 (23–54)	**<0.001**
Age first livebirth			
Mean (years) (range)	29.0 (18–40)	28.9 (18–42)	0.937
Marital Status; n (%)			
Married or cohabitating	281 (75.7)	308 (75.3)	0.682
Divorced, separated, or widowed	43 (11.6)	33 (8.1)	0.088
Single	43 (11.6)	68 (16.6)	**0.051**
Data missing	4 (1.1)	0	-
Highest Education level achieved; n (%)			
Did not complete secondary school	36 (9.7)	21 (5.1)	**0.014**
Completed secondary school	61 (16.4)	73 (17.9)	0.616
Apprenticeship/certificate	84 (22.7)	88 (21.5)	0.689
Degree	106 (28.6)	144 (35.2)	**0.050**
Postgraduate	82 (22.1)	82 (20.1)	0.469

Characteristic	Treated (N=371)	Untreated (N=409)	P-value
Data missing	2 (0.5)	1 (0.2)	-
Family income ($)			
0–25,999	35 (9.4)	40 (9.8)	0.996
26,000–51,999	78 (21.0)	85 (20.8)	0.736
52,000–77,999	71 (19.1)	88 (21.5)	0.560
78,000–103,999	67 (18.1)	83 (20.3)	0.576
104,000 +	88 (23.8)	91 (22.2)	0.446
Data missing	32 (8.6)	22 (5.4)	-
Smoking			
Ever smoked	195 (52.6)	213 (52.1)	0.769
Data missing	4 (1.1)	0	-
Oral contraceptive use			
Ever used	352 (94.9)	390 (95.4)	0.896
Data missing	2 (0.5)	1 (0.2)	-

Treated and untreated women differed in their predicted final height and their actual final height (**Table 4.2**). Specifically, more untreated women than treated women had an estimated mature height ≤177.0 cm while more treated than untreated women had an estimated mature height ≥183.0 cm. Treated women were taller than untreated women despite being treated in adolescence with high-dose estrogens to reduce their final height.

Table 4.2: Growth characteristics of treated and untreated participants.

Characteristic	Treated n (%) (N=371)	Untreated n (%) (N=409)	P-value
Estimated mature height (cm)			
≤177.0	70 (18.9)	292 (71.4)	**<0.001**
177.1–182.9	175 (47.2)	97 (23.7)	**<0.001**
≥183.0	118 (31.8)	12 (2.9)	**<0.001**
Data missing	8 (2.2)	8 (2.0)	-
Adult height (cm)			
≤177.0	109 (29.4)	200 (48.9)	**<0.001**
177.1–182.9	211 (56.9)	174 (42.5)	**<0.001**
≥183.0	50 (13.5)	35 (8.6)	**0.027**
Data missing	1 (0.3)	0	-

The groups also differed in 'ever use' of exogenous sex hormones for reproductive conditions: treated 146 (39.4%), untreated 124 (30.3%) (p=0.008). This observation is due to the greater use of fertility drugs in treated women, as reported previously[25]. In addition, treated women n=248 (66.9%) were significantly less likely to have ever had a livebirth than untreated women n=267 (65.3%): age-adjusted RR 0.87 (95% CI: 0.79 to 0.95), though this difference was small[25]. The reasons for 'ever using' sex hormones for reproductive conditions are summarised in **Table 4.3** below. Treated women were, on average, older than untreated women. This is likely to explain the greater proportion of treated women using hormone treatment for menopause and heavy bleeding (during the perimenopausal period). However these differences were not statistically significantly.

Table 4.3: Use of hormones for reproductive conditions in treated and untreated women.

Reasons for other hormone use (ever used)	Treated n (%) (N=371)*	Untreated n (%) (N=409)*	P-value
Cramps/backache associated with periods	37 (10.0)	52 (12.7)	0.240
Irregular menstrual cycles	47 (12.7)	41 (10.0)	0.232
Premenstrual tension	11 (3.0)	20 (4.9)	0.174
Heavy or prolonged menstrual bleeding	45 (12.1)	36 (8.8)	0.121
Absence of period apart from pregnancy, breastfeeding or the pill	17 (4.6)	10 (2.4)	0.100
Menopause	23 (6.2)	15 (3.7)	0.097
Other menstrual problems	8 (2.2)	10 (2.4)	0.798
Infertility	68 (18.3)	34 (8.3)	**0.0001**

* 3 missing treated, 1 missing untreated.

4.3.2 Side effects of treatment

Side effects reported at or around the same time as treatment included increased pigmentation of the nipple, breast pain, breast lumps, dry cracked/bleeding nipples, and galactorrhea either during treatment or immediately following treatment. Dry cracked/bleeding nipples arose from the 'other' side effect question in the postal questionnaire.

Overall, the reports of side effects were more common for women treated with DES compared to EE. Breast related side effects were reported by 40% of treated women: 91(59.9%) (95% CI: 51.9 to 67.3%) DES treated women and 56 (26.3%) (95% CI: 20.8 to 32.6%) EE treated women (p=0.0001), in the postal questionnaire. More DES treated women

than EE treated women experienced increased pigmentation of the nipple and galactorrhea (**Table 4.4**). As galactorrhea might be a result of sudden withdrawal of medicine, all the women who experienced galactorrhea during treatment had stated that they took their tablets regularly.

The exclusion from the analysis of women who self-referred to the study made little difference to the percent of women reporting breast related side effects (DES 57.5%, EE 23.8%, and all treated women 36.6%).

Table 4.4: Short-term side effects on the breast by drug type.

Side Effect	DES n (%) (N=152)*	EE n (%) (N=213)*	P-value	Total n (%) (N=398)†
Increased pigmentation of the areolae and nipple	81 (53.3)	43 (20.2)	**0.0001**	134 (33.7)
Breast pain	16 (10.5)	14 (6.6)	0.175	32 (8.0)
Breast lump	3 (2.0)	4 (1.9)	0.948	8 (2.0)
Dry cracked/bleeding nipples	3 (2.0)	1 (0.5)	0.191	4 (1.0)
Galactorrhea during treatment	10 (6.6)	3 (1.4)	**0.009**	14 (3.5)
Galactorrhea immediately following treatment ‡	12 (7.9)	8 (3.8)	0.087	21 (5.3)

*Does not include those treated with both DES and EE (n=5) or drug type unknown (n=28).

†Includes those treated with both DES and EE (n=5) and drug type unknown (n=28).

‡ 9/21 who experienced galactorrhea following treatment also reported experiencing it during treatment.

4.3.3 Risk of breast biopsy, surgery and cancer

The age-adjusted relative risks for ever having a breast biopsy, other breast surgery and breast cancer are described in **Table 4.5**. While 2% of treated women reported having had experienced breast lumps as a side effect of treatment, treated women were no more likely to have ever had a breast biopsy (n=45, 12.1%) than untreated women (n=46, 11.3%) (age adjusted RR 0.95, 95% CI: 0.6 to 1.4).

Risk of elective or non-elective surgery was not significantly greater for treated women. The majority of surgical procedures involved a lumpectomy (n=27, 77.1%) of which, 14 treated women (3.8%) and 13 untreated women (3.2%) had had this procedure (age adjusted RR 1.10 (95% CI: 0.5 to 2.4). Other types of non-elective surgery included: papilloma removed (n=2), breast abscess at six months of age (n=1), removal of cancer (n=1), breast abscess drained (n=2), removal of ingrown hair (n=1). Two treated women, and no untreated women had a mastectomy.

Cosmetic or elective surgeries (n=18) included: mole excised (n=1), birthmark removed (n=1), breast enlargement/implants (n= 9), reduction (n= 4), removal of extra nipple (n=2), and one unknown. There were four breast cancer cases in the treated group and two in the untreated group (age adjusted RR 1.15, 95% CI: 0.2 to 7.2). Five of six of these cases were verified with medical records.

Table 4.5: Age adjusted relative-risks of ever having had a breast biopsy, surgery, and breast cancer.

	Treated n (%) (N=371)	Untreated n (%) (N=409)	Age adjusted RR (95%CI)
Breast biopsy	45 (12.1)	46 (11.3)	0.95 (0.6 to 1.4)
Other breast surgery			
Elective	8 (2.2)	10 (2.4)	0.86 (0.3 to 2.2)
Non-elective	20 (5.4)	15 (3.7)	1.32 (0.7 to 2.6)
Breast cancer	4 (1.1)	2 (0.5)	1.15 (0.2 to 7.2)

4.3.4 History of mammographic screening

In each age decade grouping, treated women had slightly lower rates of ever having had a mammogram compared with untreated women except in the 40–49 year age group (**Table 4.6**) (**Figure 4.2**). While treated women had a higher overall proportion of ever having had a mammogram (39.9%) compared with untreated women (32.0%) (p=0.014), after adjusting for age the difference did not remain (p=0.98). More untreated women had had a mammogram in the 18–29 and 50–59 year age groups compared with ABS 2001 population rates, though numbers are too small to be confident in this finding.

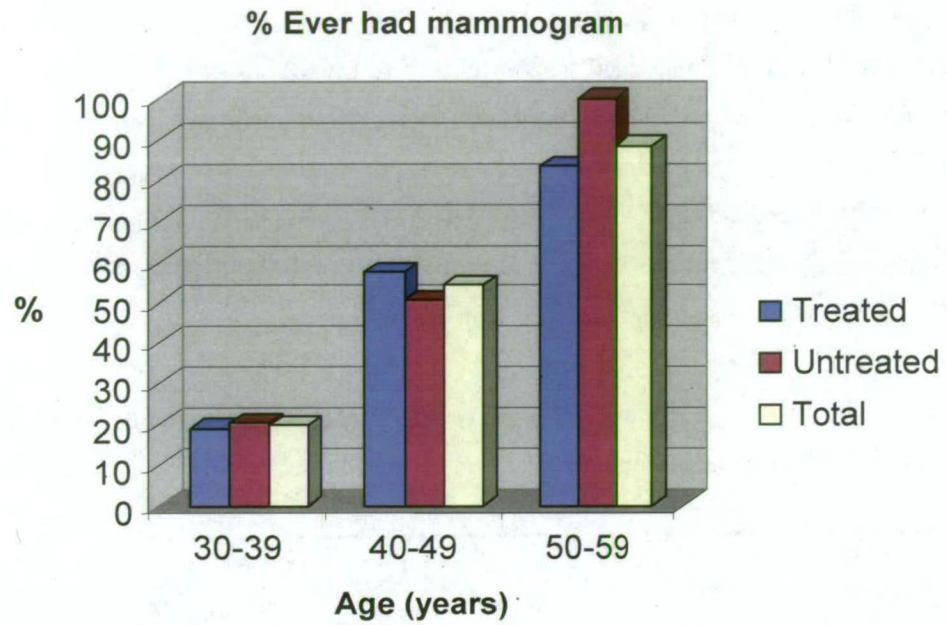
Table 4.6: Ever had a mammogram for treated and untreated and Australian population (ABS 2001) by age group.

Ever had a mammogram	Treated n (%) N=371*	Untreated n (%) N=409*	ABS† %
18–29 years	1 (3.6)	3 (8.6)	3.8
30–39 years	26 (18.4)	45 (20.5)	18
40–49 years	96 (56.1)	73 (50.7)	49.4
50–59 years	25 (83.3)	10 (100)	78.1
Total n (%)	148 (39.9)	131 (32.0)	

* Missing n=9 treated, n=4 untreated.

† Source: Australian Bureau of Statistics 2001 National Health Survey Summary of Results (p 92).

Figure 4.2: Proportion of women who had ever had a mammogram.



The reasons for having the most recent mammogram are summarised in **Table 4.7**. Reasons were similar between treated and untreated groups except that more untreated women had their most recent mammogram because they experienced symptoms, while more treated than untreated women had their most recent mammogram because of a family history of breast cancer, however these differences were not statistically significant.

Table 4.7: Reasons for last mammogram in treated and untreated women.

Reason for last mammogram	Treated n (%) N=148	Untreated n (%) N=131	P-value
Symptoms present	50 (33.8)	51 (39.9)	0.300
Family history of breast cancer	25 (16.9)	14 (10.7)	0.153
Had breast cancer in the past	4 (2.7)	1 (0.8)	0.231
General check up	44 (29.7)	38 (29.0)	0.983
Don't know	1 (0.7)	1 (0.8)	0.919
Other	19 (12.9)	19 (14.5)	0.635
Missing	5 (3.7)	7 (5.3)	-

4.4 Discussion

Short-term side effects reported by treated women included breast lumps, galactorrhea, breast pain, dry cracked or bleeding nipples and increased pigmentation of the nipple. These effects, apart from dry cracked and bleeding nipples, have been reported elsewhere[3, 22-24]. It is possible that the reports of cracked nipples were associated with nipple hyperkeratosis which is known to be associated with estrogen treatment in humans[235] and animals[236]. The only mention of hyperkeratosis in relation to estrogen treatment for tall stature was by Zackman et al. (1975)[46] and Drop et al.(1998)[44], where they mentioned that diethylstilbestrol (DES) more than other estrogen preparations has the clear disadvantage of causing marked pigmentation and hyperkeratosis of the nipples. This is odd because, unlike the former side effect of marked pigmentation, hyperkeratosis is not reported as an observed side effect for any of the subjects in the case-series report by Zackman et al. (1975)[46].

Increased pigmentation of the nipple was the most frequently reported breast related side effect. The greater proportion of DES treated women than ethinyl estradiol (EE) treated women experiencing this side effect was not unexpected and has been reported elsewhere[41].

The second most frequently reported breast related side effect was breast pain, with 8% of all women experiencing breast pain during treatment. Weimann reported breast discomfort in 6% of women treated with conjugated equine estrogens[22]. Of interest is the positive association observed elsewhere between breast pain or tenderness and breast cancer risk. Cyclical mastalgia is reported to be sensitive to estrogen and a marker of breast susceptibility to estrogen and breast cancer risk. In their case-control study, Plu-Bureau et al. (1992)[110] observed an increasing risk of developing breast cancer with increased duration of cyclical mastalgia (RR 1.12 for 6–48 months, 2.24 for 49–96 months, and 5.54 for 97 months; P for trend=0.001). A later study by Crandall et al. (2009)[237] analysed data from the WHI Estrogen and Progestin randomised controlled trial involving 8,506 women treated with conjugated estrogens and a progestin and 8,102 with placebo. They found significantly more women treated with E & P experienced new-onset breast tenderness (NOBT) compared with the placebo group, and that breast cancer risk was greatest in those treated with E & P who experienced NOBT compared to those who did not (HR 1.48; 95% CI: 1.08 to 2.03).

Tamoxifen, an anti-estrogen, has been used for treatment of breast pain (mastalgia)[238] and conclusions following a meta-analysis suggest it to be the drug of choice[239]. Another anti-estrogen (centchroman) has since been recommended as another option for the treatment of breast pain[240]. These findings support the association between breast pain, estrogen exposures and breast cancer risk. Of interest is the positive association observed between new-onset breast pain or discomfort and mammographic density after hormone therapy[241-243]. Mammographic density is a well established risk factor of breast cancer[4]. It is possible that women who experienced breast pain following treatment with high-dose estrogens in adolescence also experienced an increase in mammographic density. Mammographic density is examined in later chapters of this thesis.

In this study, breast lumps, observed in 2.0% of DES treated women and 1.9% of EE treated women, did not differ by treatment type. These figures are slightly greater than that observed by Trygstad[23] who had observed benign fibroadenoma in 0.3% of DES treated girls. It is possible that breast lumps retrospectively reported by women in this study included benign forms other than fibroadenoma.

It appears that galactorrhea, both during treatment and following treatment, occurred in more DES treated girls than EE treated girls. No other studies differentiate between treatment type and galactorrhea prevalence. Rates varied between 0%[22] to 14%[43] across all studies. In the study by Kuhn et al. (1977)[43], 14% of treated girls had experienced galactorrhea as a side effect of treatment. The estrogen used by the girls in this study was ethinyl estradiol and the dose was 500 μg/day; much greater than that used by treated girls in this study.

Galactorrhea occurred during treatment or immediately following treatment. None of the case-series reports reviewed differentiated between the two distinct occurrences. It is possible the side effect is due to increased prolactin levels which have been observed in studies of treated girls[13]. According to Chatterton[98], the positive feedback of estrogen on prolactin secretion provides a stimulus for breast development. Withdrawal of the steroid, particularly if prolactin levels remain elevated for any reason, may precipitate galactorrhea.

Galactorrhea has been associated with the cessation of oral contraceptive use in a case-control study[244].

It is uncertain why galactorrhea was observed in girls during treatment. This observation may be associated with poor compliance to treatment resulting in a sudden drop in estrogen levels and therefore prolactin levels, though this is unlikely because all women who had reported that they had experienced galactorrhea during treatment had also reported that they had taken their medication regularly. Alternatively, it is possible that the increased prolactin levels that occur in some treated girls may be transient and drop back to normal levels or below normal levels while still undergoing treatment. This has been observed elsewhere[13].

While reports suggest that some girls have experienced incomplete breast development as a result of treatment[23, 41], no women in this study reported this to be a side effect of treatment. It is a subjective measure, and it is unlikely that women noticed cessation of growth unless there was a reduction or regression in breast volume with treatment. Total breast area is examined in a later chapter as a necessary measure for the calculation of percent mammographic density and could provide additional information about breast size differences between treated and untreated women.

It appears from the papers reviewed that this is the first study to quantify the rates of breast related side effects for different treatments from the same cohort of women. Overall, DES treated women reported more breast related side effects (59.9%) than EE treated women (26.3%), with the increase largely due to the different rates for galactorrhea and pigmentation of the nipple.

Treatment with high-dose estrogens to reduce the adult height of tall girls was associated with short-term side effects on the breast but did not appear to be associated with breast disease in the long-term. There was no significant difference between treated women and untreated women in having ever had a breast biopsy, breast surgery (including lumpectomy), or a diagnosis of breast cancer.

Mammographic screening history was collected to determine the degree and direction of ascertainment bias that might have existed due to non-random sampling of the treatment and untreated groups. Women who have a mammogram are more likely to have a lump or abnormal tissue identified in the screening process, leading to a breast biopsy, diagnosis of benign breast disease or breast cancer. It is possible that fewer treated than untreated women had ever had a mammogram, picking up fewer cases of breast lumps or tissue abnormalities requiring a breast biopsy, and diagnoses of breast cancer, or vice versa. Age adjusted rates of mammographic screening did not differ between treated and untreated women.

Treated women were no more likely than untreated women to have had their most recent mammogram because of the presence of symptoms. This is consistent with the finding that treated women were no more likely to have had a breast biopsy, or breast cancer than untreated women.

It is also likely that women who had their most recent mammogram because of a family history of breast cancer, themselves had a higher risk of breast cancer and as a consequence of the screening, had breast cancer detected, albeit earlier, before symptoms presented. However, treated women were no more likely to have had their most recent mammogram because of a family history of breast cancer than untreated women.

One limitation of this study is the reliance on self-reporting by women. In relation to the short-term side effects of treatment, women were asked to recall events that occurred between 10 and 40 years earlier. The self-reporting of side effects of treatment could result in under-reporting of minor breast ailments. For instance, the prevalence of breast lumps occurring during treatment may be under-represented. Breast lumps can be painful; however, if not painful, the presence of a lump may not have been known. Even for those symptoms that were known at the time, they may not be recalled if they were not too unpleasant. On the other hand, recall bias might result in the over-reporting of breast ailments. Treated girls might have been more watchful of breast changes and symptoms during treatment and readily attributed these changes to treatment. However, a difference was observed in only some breast related side effects between the two groups. This suggests that recall bias is unlikely.

History of breast biopsies and breast surgery were more likely to be remembered by participants compared with reporting a diagnosis of benign breast disease or a breast lump, and evidence of a clinical investigation was considered important for a diagnosis. However, the number of breast biopsies should not be seen to accurately represent the prevalence of benign breast disease in this study. A breast biopsy is performed to partially or fully extract a suspicious breast lump and perform a diagnostic test on the tissue. The abnormal tissue is then diagnosed as either benign, or cancerous. Not all women with benign breast disease are symptomatic. Asking women if they ever had a breast biopsy will only identify those women whose lumps were brought to notice through self examination, a doctor's clinical examination or mammogram.

In addition, not all identified lumps are biopsied. The doctor may request an ultrasound or MRI to first rule out a breast cyst (fluid filled sac). If it is a solid mass, a breast biopsy or breast surgery to remove the lump may be performed. Therefore, asking if a woman has had a breast biopsy is unlikely to pick up breast cysts. Findings to the question 'have you ever had a breast biopsy' will only be representative of biopsy-demonstrated benign breast disease (excluding those who were diagnosed with cancer or found to have no abnormality at all). However, if treated women had more cases of breast disease than untreated women, it would be expected that they would have had more breast biopsies for a given age.

At the start of this study, it was recognized that the cohort size was too small, and participants relatively too young, to expect to pick up many cases of breast cancer, and that it was not suitable for a reliable assessment of the level of breast cancer risk associated with high-dose estrogen treatment in tall girls. However, it was also recognised that the cohort could provide some indication of the incidence in the two groups of women, and whether the rates for treated women appeared out of the ordinary. As anticipated, the numbers of breast cancer cases were small for both groups, and the rates did not appear out of the ordinary. In Australia, the 20 year prevalence rate for women 35-39 years of age in 2006 was 1.6%. This prevalence rate is based on the number of surviving persons who received a breast cancer diagnosis in the last 20 years[245]. The mean ages of women in the Tall Girls Study were 39.7 years (treated) and 37.7 years (untreated) at the time that they were asked about their breast cancer history. Assuming there was no increased or

decreased risk of breast cancer in these women and based on the population prevalence rates, it would be expected that at least 1.6% of women would have had a diagnosis of breast cancer previously. In this cohort, the rate was 1.1% for treated and 0.5% for untreated.

A larger sample size and a longer follow-up period would be needed to better investigate breast cancer risk. For instance, with the existing sample size, at least 6% of treated or untreated women would need to have had breast cancer for the study to have 80% power to detect a significant difference between the two groups (assuming the proportion for the other group was similar to the Australian population prevalence of 1.6%).

A larger sample size and more systematic collection of breast cancer risk factors would also provide the opportunity to perform multivariable analyses to address any systematic differences between treated and untreated women that might explain any association between breast disease and treatment with high-dose estrogens in adolescence. A prospective or randomised controlled trial, while stronger in design, would not be possible. The practice of treating tall girls with high-dose estrogens is no longer common, and unethical given the adverse effects on fertility that have been reported previously[25].

4.5 Conclusion

This study identified a number of breast related side effects of treatment and found some were more prevalent among women who were treated with diethylstilbestrol compared with ethinyl estradiol. These effects are likely to have caused significant discomfort and, in some cases, embarrassment in treated girls. Treated women might be concerned about these findings and the potential for longer term effects on the breast. Reassuringly for treated women, this investigation found no significant difference between treated women and untreated women in having ever had a breast biopsy, breast surgery (including lumpectomy), or a diagnosis of breast cancer.

An alternative measure of breast cancer risk is mammographic density. This was examined in follow-up 2 as part of this PhD research study, and the findings are presented in Chapters 7 and 8. Prior to this, the findings in relation to the effect of treatment with high-dose estrogen in adolescent girls for tall stature on breast function are presented in Chapter 5.

Box 4.1: Summary of key chapter 4 findings.

KEY FINDINGS: CHAPTER 4

- Short-term breast related side effects of treatment included breast lumps, galactorrhea, breast pain, dry cracked or bleeding nipples and increased pigmentation of the nipple.
- Short-term side effects of treatment were more frequently reported in women treated with diethylstilbestrol compared with those treated with ethinyl estradiol.
- There was no significant difference between treated women and untreated women in having ever had a breast biopsy, breast surgery (including lumpectomy), or a diagnosis of breast cancer.
- The cohort size was too small and follow-up period too short to pick up sufficient cases for a reliable assessment of the level of breast cancer risk associated with high-dose estrogen treatment in adolescence. However it has provided some indication of the difference in the rates between the two groups of women.

5: TREATMENT WITH HIGH-DOSE ESTROGENS IN ADOLESCENCE AND SUBSEQUENT EFFECTS ON LACTATION

5.0 Introduction

An important finding in Chapter 4 is that study participants treated with estrogens for tall stature reported a range of short-term side effects on the breast, confirming similar findings in the literature. When this information is considered together with the reported changes in the hormonal milieu of girls treated with estrogens described in Chapter 2, and previous findings from the Australian Tall Girls Study on the long-term effects of treatment on fertility, it seems possible that treatment could have long-term effects on breast histology and therefore, function.

This chapter describes the part of this PhD study that investigated the long-term effects of treatment with high-dose estrogen on lactation, in particular breast feeding commencement and duration, in the Australian Tall Girls Study cohort.

5.1 Study Aim

The aim of this chapter is to use data collected in follow-up 1 of the Australian Tall Girls Study to 1) study the effect of treatment on breastfeeding commencement by comparing breastfeeding initiation rates between treated and untreated women, 2) compare breastfeeding duration in treated and untreated women, 3) compare the reasons for stopping breastfeeding between treated and untreated women, 4) and compare the rates of pregnancy induced breast enlargement in treated and untreated women.

5.2 Methods

This section presents the methodology used to collect and analyse the data, the findings; and finally, the discussion and conclusion.

5.2.1 Participants

As described in Chapter 4, participants included women who obtained a medical opinion about their tall stature and had radiological assessment of their skeletal age during adolescence. They included women who received estrogen treatment (3mg DES or 150 μg EE daily) in adolescence to reduce their adult height (treated group) and women who had not (untreated). Data on reproductive and breastfeeding history (those who consented to the CATI) were available for 371 treated (72% of those traced) and 409 (56%) untreated women. Recruitment strategies and responses are described more fully in Section 4.2.2. The collection of exposure data from medical records is described more fully in Section 4.2.3.

5.2.2 Measures of breastfeeding

The breastfeeding questions asked in the computer assisted telephone interview are presented in **Figure 5.1** below.

For every livebirth, women were asked if they commenced (initiated) breastfeeding their baby, and if they did, how long their baby was breastfed with breast-milk only, how long they breastfed their baby altogether (including when this baby had formula &/or solids), and the reason why they stopped breastfeeding the baby. If they did not commence breastfeeding, they were asked the reason for not commencing breastfeeding. Duration of breastfeeding was expressed in days, weeks or months in the questionnaire, and converted to weeks.

Figure 5.1: The breastfeeding questions asked in the computer assisted telephone interview (set up for 8 babies).

Now I would like to ask you some questions about **BREASTFEEDING**, starting with your first child.

	C8. DID YOU COMMENCE BREASTFEEDING THIS BABY?	C9. How long did your baby have breast milk only?	C10. How long, did you breastfeed this baby all together (including when this baby had formula &/or solids)	C11a. Why did you stop breastfeeding this baby? *(Ask as an open ended question and enter numbers that apply)* **See Table C11b**	C12a. If you didn't commence breastfeed this baby –what was the reason? (*Ask as an open ended question and enter numbers that apply*) **See Table C12b**	C13. Did you notice that your breast increased in size while pregnant?
BABY 1	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐Days ☐☐ Weeks ☐☐Months ☐ currently b'feeding	ENTER NUMBER(s) ☐,☐,☐☐ →go to C13	ENTER NUMBER(s) ☐,☐,☐☐ →go to C	YES ☐ NO ☐
BABY 2	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	ENTER NUMBER(s) ☐,☐,☐☐ →go to C13	ENTER NUMBER(s) ☐,☐,☐☐ →go to C	YES ☐ NO ☐
BABY 3	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	ENTER NUMBER(s) ☐,☐,☐☐ →go to C13	ENTER NUMBER(s) ☐,☐,☐☐ →go to C	YES ☐ NO ☐
BABY 4	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	ENTER NUMBER(s) ☐,☐,☐☐ →go to C13	ENTER NUMBER(s) ☐,☐,☐☐ →go to C	YES ☐ NO ☐
BABY 5	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	ENTER NUMBER(s) ☐,☐,☐☐ →go to C13	ENTER NUMBER(s) ☐,☐,☐☐ →go to C	YES ☐ NO ☐

Figure 5.1 continued.

C11b Reason why stopped breastfeeding

1. Didn't want to breastfeed or didn't want to breastfeed any longer
2. Nipple trauma
3. Nipple pain
4. Felt there was not enough milk
5. Unable to get baby to attach/suck/difficulties attaching the baby to the breast
6. Baby very premature
7. Lack of help/support/supervision with breastfeeding
8. Mastitis
9. Recurrent mastitis
10. Baby had poor weight gain
11. Advice from professional – who (please state e.g. GP, psychiatrist)
12. Employment reasons
13. Baby lost interest/always looking around/stopping & starting feeding
14. Breasts didn't fill or became engorged in first few days
15. Other (please specify).....

C12b Reason why never attempted to breastfeed baby

1. Did not want to breastfeed
2. Wanted to bottle feed
3. Employment/work reasons
4. Partner preferred me to bottle feed
5. Family preferred me to bottle feed

These questions had been used previously in a study of attachment to the breast and family attitudes to breastfeeding[246] where the interview questions were piloted extensively and modified before use[247]. Reasons for stopping breastfeeding were asked of women to separate physiological (e.g. nipple trauma, nipple pain, mastitis) and convenience (e.g. employment[248]) reasons for stopping breastfeeding. An 'Other – please specify' option was listed for those responses that did not fit any of the reasons listed. If treatment did influence lactation the reasons for stopping breastfeeding would be expected to be physiological or due to inadequate nutritional support of the baby. These explanatory questions therefore validate any associations observed between treatment and breastfeeding duration.

Women were asked whether they noticed their breasts increase in size while pregnant. The question on breast changes during pregnancy was asked to ascertain whether there was mammary development during pregnancy in preparation for lactation and in response to anecdotal evidence that this may have been a problem in treated women.

5.2.3 Other covariates

Parity has been positively[249], and negatively[250] associated with breastfeeding duration in Australian studies, while breastfeeding initiation has been found to be negatively[249] associated with parity. In the CATI, women were asked how many pregnancies they had, the outcome of each pregnancy and the date when each pregnancy ended. Number of livebirths was derived from this data. Maternal age at birth, which has been shown to be positively associated with breastfeeding duration in Australian women[249-252], was derived from the date of each livebirth collected in the CATI.

Data on other potential confounders such as maternal education[251-255] and other socio-economic indicators[256] were collected in the postal questionnaire as described in Chapter 4 (Section 4.2.5).

5.2.4 Statistical methods

Stata software (version 8) was used for all statistical analysis. Chi-square tests were performed for tests of significance for categorical descriptive data, and t-tests for continuous descriptive data. All tests of significance were two-sided. The precise p-values were reported for descriptive data comparisons. Following convention, nominal statistical significance was based on a p-value less than 0.05. Relative risks and 95% confidence intervals were calculated using log binomial regression. A discussion of the choice of threshold for statistical significance is described in section 4.2.6.

For the regression analysis of breastfeeding duration data, the assumption that the errors were distributed normally was checked using normal quantile plots of the residuals. If transformation of the breastfeeding duration variable was required, the Box-Cox method[257] was used to identify the most appropriate transformation. Treated and untreated transformed data were compared using linear regression and medians and 5% and 95% percentiles reported.

Potential confounding variables (e.g. number of livebirths, age, year of birth and maternal age at first live birth) were entered into the model one at a time and retained if their presence was significant. This study examined breastfeeding initiation and duration in treated and untreated primiparous women so did not include number of livebirths in the risk estimation model. However, when breastfeeding duration was explored for all livebirths, the number of livebirths a woman ever had was included in the model.

Since fertility problems have been associated with treatment[25] statistical interaction between treatment status and maternal age at birth and number of livebirths was examined when comparing treated and untreated results for breastfeeding commencement or duration.

5.3 Results

5.3.1 Characteristics of study participants

The characteristics of study participants are shown in **Table 5.1**. The mean age of participants was 39.8 years (range 20–55) in the treated group and 37.7 years (23–54) in the untreated group (p=**<0.001**). As reported in Chapter 4, treated and untreated study participants were similar in their marital status, though slightly more treated women were single than untreated women (p=0.051). Women were similar in the highest educational level achieved in most categories, though more treated than untreated women did not complete secondary school (p=0.014) and fewer had a degree (p=0.050). Treated and untreated women were also similar in family income, smoking history, oral contraceptive use and mean age at first livebirth. As reported elsewhere[25], treated women n=248 (66.9%) were significantly less likely to have ever had a livebirth than untreated women n=267 (65.3%): age-adjusted RR 0.87 (95% CI: 0.79 to 0.95), though this difference was small.

5.3.2 Treatment characteristics

Of the treated women completing the CATI, 146 (39.4%) used DES, 195 (52.6%) used EE, five (1.3%) used both and the estrogen type was missing for 25(6.7%). Women were treated for a mean duration of 24.7 months and commenced treatment at a mean age of 12.8 years. Treatment commenced before menarche in 52.9% of treated girls. First assessment and hence treatment occurred at or after Tanner stage 3 (breast development) for 51% (54.8% DES, 49.0% EE) of treated girls. In addition to estrogen, a progestagen was routinely administered as 5 mg twice daily for 4 days every month to induce cyclical bleeding.

Table 5.1: Characteristics of study participants.

Characteristic	Treated (n=371)	Untreated (n=409)
Age		
Mean (years)	39.8	37.7
Age first livebirth		
Mean (years)	29.0	28.9
Marital Status n (%)		
Married or cohabitating	281 (75.7)	308 (75.3)
Divorced, separated or widowed	43 (11.6)	33 (8.1)
Single	43 (11.6)	68 (16.6)
Data missing	4 (1.1)	0
Highest Education Level n (%)		
Did not complete secondary school	36 (9.7)	21 (5.1)
Completed secondary school	61 (16.4)	73 (17.9)
Apprenticeship/certificate/diploma	84 (22.7)	88 (21.5)
Degree	106 (28.6)	144 (35.2)
Postgraduate	82 (22.1)	82 (20.1)
Data missing	2 (0.5)	1 (0.2)
Family Income (Aust$) n (%)		
$0–25,999	35 (9.4)	40 (9.8)
$26,000–51,999	78 (21.0)	85 (20.8)
$52,000–77,999	71 (19.1)	88 (21.5)
$78,000–103,000	67 (18.1)	83 (20.3)
$104,000+	88 (23.8)	91 (22.2)
Data missing	32 (8.6)	22 (5.4)
Smoking n (%)		
Ever smoked	195 (52.6)	213 (52.1)
Data missing	4 (1.1)	0
Oral contraceptives n (%)		
Ever used	352 (94.9)	390 (95.4)
Missing data	2 (0.5)	1 (0.2)

5.3.3 Breastfeeding commencement

Women who had been treated with estrogens to reduce their adult height were no more likely than untreated women to not commence breastfeeding following their first live birth (age adjusted RR 1.13, 95% CI: 0.50 to 2.52) or any livebirth (age adjusted RR 1.12, 95% CI: 0.43 to 2.90) (**Table 5.2**). No significant statistical interaction was observed between treatment status and maternal age at first pregnancy or number of livebirths.

Table 5.2: Breastfeeding commencement.

	Treated n (%)	Untreated n (%)	Adjusted RR* (95%CI)
Did not commence breastfeeding first livebirth	11 (4.4)	11 (4.1)	1.13 (0.50 to 2.52)
Did not commence breastfeeding any livebirth	8 (3.2)	8 (3.0)	1.12 (0.43 to 2.90)

N=248 treated, 267 untreated (those having had a livebirth)

* Adjusted for maternal age at first livebirth.

Reasons for not commencing breastfeeding the first baby included having had no milk (treated n=2, untreated n=1), non breast-related medical reasons for mother or baby (treated n=4, untreated n=2), did not want to breastfeed, preferred bottle feeding or for employment reasons (treated n=4, untreated n=4), baby was not interested (untreated n=1), or reason missing (treated n=1, untreated n=3).

5.3.4 Breastfeeding duration

In women who commenced breastfeeding, there was no meaningful difference between treated and untreated women in duration of breastfeeding in total or exclusively for the first or all livebirths (**Figure 5.2**) (**Table 5.3**).

Figure 5.2: Mean breastfeeding duration (weeks) for treated and untreated women.

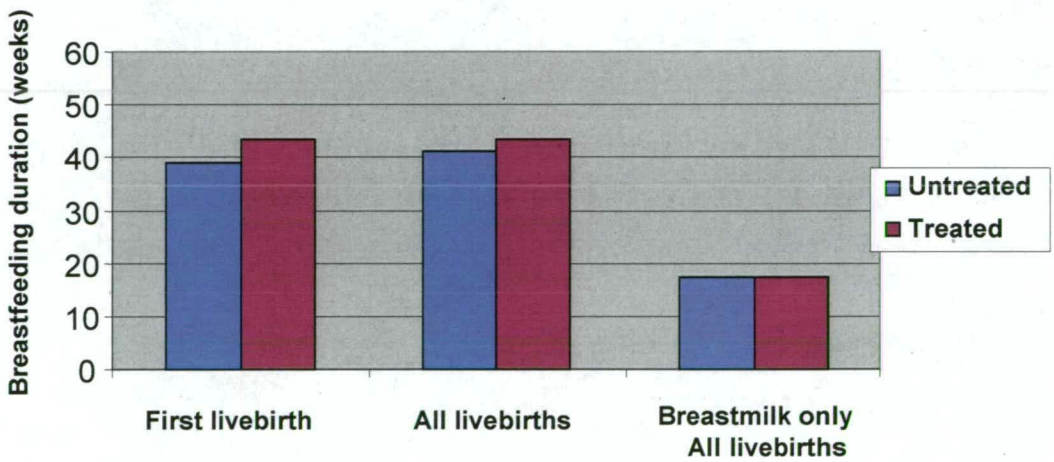


Table 5.3: Breastfeeding duration for those who initiated breastfeeding

	Treated Median (5^{th}–95^{th} percentile range)	Untreated Median (5^{th}–95^{th} percentile range)	P-value
Breastfeeding duration for first birth (weeks)*	39.0 (6.0–77.9)	43.3 (6.0–77.9)	0.77
Breastfeeding duration for all livebirths (weeks) (mean)**	41.1 (6.0–95.3)	43.3 (5.0–82.3)	0.77
Feeding duration for breastmilk only, all livebirths (weeks) (mean)	17.3 (2.3–30.3)	17.3 (1.5–28.9)	0.81

N=240 treated, 259 untreated. Missing 11 treated and eight untreated for breastfeeding duration for first and all livebirths, six treated and one untreated of breastmilk only duration. Number of women who ever breastfed is greater than number who breastfed their first livebirth.

* Adjusted for maternal age at first livebirth

** Adjusted for age at interview

The distribution of the breastfeeding duration data was not normal. The square root transformation emerged as the most suitable method for total breastfeeding duration. No transformation was required for feeding duration for exclusive breastfeeding. Maternal age at first livebirth and age was significant for duration for first livebirth and all livebirths respectively and were included in the regression model. Adding year of first livebirth to the regression did not change the result. No significant interaction effect was observed between treatment status and age of first pregnancy or number of livebirths.

Treated women who commenced breastfeeding their first baby were no less likely to be breastfeeding at four, 24 or 52 weeks than untreated women. Of those who breastfed their first baby 2.7% treated and 4.0% untreated women had stopped breastfeeding by four weeks. At 24 weeks, 75.4% untreated and 77.4% treated were continuing to breastfeed their first baby, and at 52 weeks, 24.6% untreated and 20.4% treated were continuing to breastfeed their first baby.

Women whose treatment started before menarche were not significantly more likely to stop breastfeeding earlier than women whose treatment started after menarche. Of those who commenced treatment before menarche, 1.8% stopped breastfeeding before four weeks while 3.5% of those who commenced treatment after menarche stopped before four weeks ($p=0.42$).

There was no difference in the duration of breastfeeding the first baby, in total ($p=0.18$) or exclusively ($p=0.93$), between women treated with DES and EE.

5.3.5 Reasons for stopping breastfeeding

Treated women were no more likely than untreated women to have stopped breastfeeding their first baby for physiological reasons affecting the breast (**Table 5.4**). Reasons included: feeling that there was insufficient milk or it had dried up (13.1% treated, 12.9% untreated), they had nipple pain (1.3% treated, 3.1% untreated), nipple trauma (0.9% treated, 2.3% untreated), or because of mastitis (1.7% treated, 1.6% untreated). It appeared that treated women were more likely to have stopped breastfeeding because their breasts did not fill or

become engorged (adjusted RR 4.48, 95% CI: 0.98 to 20.60) but very few women were in this category for both groups.

Table 5.4: Reasons for stopping breastfeeding for physiological reasons.

Reason for stopping breastfeeding	Treated n (%) (N=236)*	Untreated n (%) (N=256)	RR (95% CI)
Felt there was not enough milk/milk dried up	31 (13.1)	33 (12.9)	1.02 (0.65 to 1.61)
Nipple trauma	2 (0.9)	6 (2.3)	0.36 (0.07 to 1.77)
Nipple pain	3 (1.3)	8 (3.1)	0.41 (0.11 to 1.52)
Mastitis	4 (1.7)	4 (1.6)	1.08 (0.27 to 4.23)
Breasts did not fill or become engorged	8 (3.4)	2 (0.8)	4.48 (0.98 to 20.60) †

A woman may have provided more than one reason for stopping breastfeeding.

Four untreated and two treated women were still breastfeeding.

* Missing n=1 treated

† Adjusted for maternal age at first birth and number of livebirths

Treated women were no more likely than untreated women to have stopped breastfeeding for a range of non-physiological reasons that included not wanting to breastfeed any more or the baby was old enough to be weaned (adjusted RR 0.94, 95% CI: 0.74 to 1.18), poor baby weight gain (RR 0.81, 95% CI: 0.29 to 2.31), baby lost interest/weaned itself (RR 0.99, 95% CI: 0.68 to 1.44); employment reasons (RR 0.92, 95% CI: 0.50 to 1.72), next pregnancy planned or started (RR 0.91, 95% CI: 0.48 to 1.73), unable to get baby to suck/attach (RR 1.19, 95% CI: 0.52 to 2.76), and lacking confidence/support (adjusted RR 0.75, 95% CI: 0.29 to 1.93).

5.3.6 Change in breast size during pregnancy

Treated women n=12 (4.9%) were no more likely than untreated women n=15 (5.7%) to have reported that their breasts did not increase in size during their first livebirth pregnancy (RR 0.87, 95% CI: 0.41 to 1.82) or for any livebirth pregnancy (7.0% treated, 7.6% untreated).

Of the women who reported that they stopped breastfeeding because their breasts did not fill or become engorged after their first livebirth, (eight treated, two untreated), six of the treated and all of the untreated had reported that their breasts had increased in size during that pregnancy.

Of the women who did not commence breastfeeding their first baby because they did not produce milk (two treated, one untreated), all had reported that their breasts increased in size during pregnancy.

5.4 Discussion

This is first study to examine the long-term effects of estrogen treatment for tall stature on lactation. This study found no meaningful differences in breastfeeding commencement rates or breastfeeding duration, between women treated with high-dose estrogens during adolescence and untreated women. This is despite women reporting short-term side effects on the breast as described in Chapter 2.

As previously reported, women with insufficient glandular tissue of the breast are known to suffer from lactation failure and a lack of breast enlargement during pregnancy[230, 258]. Complaints of flat-chestedness by girls treated with estrogens for tall stature have been reported[23, 41]. It is possible that the reduced IGF-I levels observed with treatment[11] could increase the risk of breast hypoplasia, and consequently a lack of breast enlargement during pregnancy and lactation insufficiency. However, in this study, women treated with estrogen during adolescence for tall stature were no more likely to report a lack of breast enlargement during pregnancy, or lactation insufficiency, than untreated women.

In addition, a lack of breast engorgement or filling of the breasts did not seem to be associated with breast hypoplasia because most of the women reporting a lack of breast engorgement or filling of the breasts reported an increase in breast size during pregnancy. There was no difference in the number of treated and untreated women who reported that their breasts did not increase in size during any pregnancy.

A strength of this study was the collection of data on the reasons for stopping breastfeeding. If treatment affected breastfeeding duration it would be expected that more treated women would have stopped breastfeeding because of difficulties with it. The reasons for stopping breastfeeding were similar between treated and untreated women. Treated women were no more likely to have stopped breastfeeding because of a lack of milk, nipple trauma or pain, or mastitis. More treated than untreated women reported stopping because their breasts did not engorge or fill with milk but the numbers were small for both groups.

One limitation of the study is that women were asked to recall their breastfeeding history on average 10.7 years (range 0.4–33 yrs) after breastfeeding their first livebirth. A review of 11 studies that examined the reliability and validity of breastfeeding recall data suggests that maternal recall is a valid and reliable estimate of breastfeeding initiation and duration, but is less satisfactory for exclusive breastfeeding[259]. Tomeo *et al.* (1999)[260], for instance, observed a high reliability of long-term recall of breastfeeding duration (r=0.86). Two Australian studies support the use of maternal recall data 10–15 years after the event[261] [262]. Tienboon *et al.* (1994)[261] reported that one third of women in their study recalled breastfeeding duration with a one month accuracy, while 59% recalled it accurately within two months, 14–15 years post the breastfeeding event. On average, mothers overestimated duration by 1.3 months[261].

A later study examined the recall accuracy of reasons for weaning by comparing data collected by interview soon after breastfeeding with data collected years later from the same women, and found varied results depending on the reason (54% sensitivity for nipple cracks/sores, 84% for mastitis) 1.3–5 years following birth[263].

In 1991, The World Health Organization[264] developed a definition of exclusive breastfeeding that only allows breastmilk to be received from the mother, a wet nurse, or expressed, and no other liquids or solids with the exception of drops or syrups consisting of vitamins, mineral supplements or medicines to be taken during the period of exclusive breastfeeding. When women reported on the length of time their baby was breastfed with breast-milk only, it is possible that the babies had taken water during this period. This study is interested in the difference in breastfeeding duration (exclusively, and in total) between treated and untreated women and there is no reason why treated and untreated women would differ in their interpretation of this question. According to Chapman *et al.* (2009)[265] slight variations in wording of the breastfeeding duration questions can result in different estimates of breastfeeding duration, especially among infants receiving predominantly expressed breast milk. Women who expressed their breast milk for instance, might respond to the breastfeeding duration question in this study differently to those who received their breastmilk by the breast. However, it is unlikely that treated and untreated women would interpret the question differently.

As suggested in a study reporting DDE and PCB exposures on lactation duration and initiation[214], some mothers may have stated that they did not commence breastfeeding when in fact they had but stopped feeding in the first few days following birth. These women would have otherwise been included in the group of women who had commenced breastfeeding, and for whom breastfeeding duration was calculated. If this occurred, the number of women who did not initiate breastfeeding would be lower than reported, and the number of women with a shorter duration of breastfeeding would have been larger than reported[214]. If misclassification occurred as described, but equally between the treated and untreated women, then there would be no effect on the regression results, though the median number of women who commenced breastfeeding would be higher, and the median duration would be lower for both groups of women. It is very unlikely that this form of misclassification was greater in one group compared with the other.

As well as maternal age and parity, which were included in the risk estimation models in this chapter, maternal education[251-255] and other socio-economic indicators[256], smoking[252, 255, 266], and BMI[255, 267, 268] at time of birth of offspring have also been shown to be associated (inversely) with breastfeeding duration. Mothers with greater levels of self-confidence[269] during pregnancy have been shown to breastfeed for longer. In their prospective cohort study of 556 Western Australian women and 503 women from Queensland, Scott *et al.* (2001)[250] found that mothers born in Australia[250] were more likely to breastfeed for longer, while Baghurst *et al.* (2007)[252] in their prospective study of 317 South Australian primiparous women, found the opposite. Similarly, breastfeeding initiation in Australian women has been positively associated with higher occupational status[249] and overall socioeconomic index for areas (SEIFA)[254], and inversely associated with BMI[267], being Australian-born, unmarried, and current smoking[270].

Treated and untreated women were similar overall in educational and marital status and level of family income, and the majority of treated and untreated women were born in Australia. These factors were not included in the multivariable model for breastfeeding duration or initiation. Data on BMI and smoking during pregnancy or at time of birth was not collected in this study. There is no clear evidence to suggest that smoking rates differed between treated and untreated women during pregnancy, as current rates of smoking were

similar between the groups. As well as self-confidence reported above, longer breastfeeding duration has been associated with lower levels of anxiety and depression; increased self-esteem and coping capacity, and stronger social health in Australian women[255, 271]. An examination of the long-term psychosocial outcomes of the Australian Tall Girls Study cohort using the depression, mania and eating disorders modules of the Composite International Diagnostic Interview (CIDI), the SF-36, and an index of social support; observed higher levels of depression in both treated and untreated women compared to the findings of population based studies[54]. There was no difference in psychosocial outcome between treated and untreated women.

Animal studies suggest that estrogen exposure during the period that corresponds to allometric growth of the mammary gland in humans can exert histological and functional effects on the mammary gland. Long-term effects on lactation were not demonstrated in this study of women exposed to high-dose estrogens during adolescence. We need to be cautious when comparing species in relation to such exposures. The typical time period between puberty and gestation in many laboratory and domestic animals is short, making comparisons with humans difficult. Estrogen induced changes to the mammary gland in animals during the prepubertal or pubertal period may not have had time to recover before the occurrence of gestation and lactation. For example, in the study on heifers reported earlier, calving occurred 12.5 months following the end of pre-pubertal treatment with estrogen implants[27]. In our study, the mean time between the end of treatment and first birth was 15.7 years.

While recognising the need for caution when comparing species in relation to exposures, the author contacted Herbert Allen Tucker (1936-2009), a renowned dairy scientist who had decades of research behind him in the area of mammary development and dairy lactational research, for his view on the likelihood of long-term effects of pubertal exposure to high-dose estrogen on later lactation.

His email response (a few years before his death) was as follows:

> *"I don't know of any effects of estrogen given during adolescence on subsequent long-term effects on mammary function. During the actual administration of estrogens you can be sure that mammary duct growth will be markedly increased. I would expect that following withdrawal of estrogen the gland would regress. In fact, we stimulated mammary growth in young growing heifers with melengesterol (MGA, which blocks the estrous cycle because of its estrogenic and progestin activity), then stopped the MGA to allow resumption of estrous activity, bred the animals, and measured subsequent lactational performance. Mammary growth was temporarily increased, but there was no long-term effect on milk production".* (2005)

This study supports the prediction by H. Allen Tucker.

5.5 Conclusion

While treatment with high-doses of estrogens in adolescence is associated with short-term adverse effects on the breast, in the longer term, treated women appeared to be no different to untreated women in their ability to breastfeed their babies. There was no difference between treated and untreated women in breastfeeding initiation, and duration. These findings are reassuring in light of evidence of adverse effects on other reproductive outcomes[25].

Whether treatment for tall stature has effects on mammary tissue growth in the short- and long-term is unknown. The next part of this chapter (Part C) examines the effect of high-dose estrogen treatment in adolescence on mammographic density, a well established risk factor for breast cancer, and a measure of the area of epithelial and stromal tissue in the breast.

Box 5.1: Summary of key findings: Chapter 5.

KEY FINDINGS: CHAPTER 5

- This study found no meaningful differences in breastfeeding commencement or breastfeeding duration between women treated with high-dose estrogens during adolescence and untreated women.
- Treated women were no more likely to report a lack of breast enlargement during pregnancy or lactation insufficiency than untreated women.
- The reasons for stopping breastfeeding were similar between treated and untreated women. Treated women were no more likely to have stopped breastfeeding because of a lack of milk, nipple trauma or pain, or mastitis. More treated than untreated women reported stopping because their breasts did not engorge or fill with milk but the numbers were small for both groups.
- A lack of breast engorgement or filling of the breasts did not seem to be associated with breast hypoplasia because most of the women reporting a lack of breast engorgement or filling of the breasts reported an increase in breast size during pregnancy.

PART C

Chapter 6: Current evidence of associations between estrogen exposures and mammographic density, a risk factor of breast cancer

Chapter 7: The long-term effect of high-dose estrogen exposure in adolescent girls on mammographic density

Chapter 8: Childhood and adolescent growth parameters and mammographic density in treated and untreated women

6: CURRENT EVIDENCE OF ASSOCIATIONS BETWEEN ESTROGEN EXPOSURES AND MAMMOGRAPHIC DENSITY, A RISK FACTOR OF BREAST CANCER

6.0 Introduction

Chapter 3 presented strong evidence of an association between hormonal exposures and breast cancer risk. Studies of hormonal exposures during periods of intense mammary development demonstrate evidence of an association with breast cancer risk. It is plausible then, that girls treated with high-dose estrogens during adolescence have an increased risk of breast cancer later in life. Breast cancer cases in the Tall Girls Study cohort were examined in Chapter 4. However, the size of the cohort was too small to pick up sufficient cases for a reliable assessment of the level of breast cancer risk associated with high-dose estrogen treatment in tall girls. An alternative measure is mammographic density, a marker of breast cancer risk.

This chapter begins by describing mammographic density and the different methods of density measurement, and then follows with a review of studies that have examined associations between mammographic density and breast cancer risk. It explores the evidence of hormonal influences of mammographic density (both exogenous and endogenous) to support the suggestion that hormone use in adolescence could possibly affect mammographic density. Evidence of associations between adolescent exposures and mammographic density later in life is examined. Evidence presented in this chapter, when viewed together with the findings reported previously, highlights the plausibility that adolescent exposure to high-dose estrogens may lead to changes in mammographic density in adulthood.

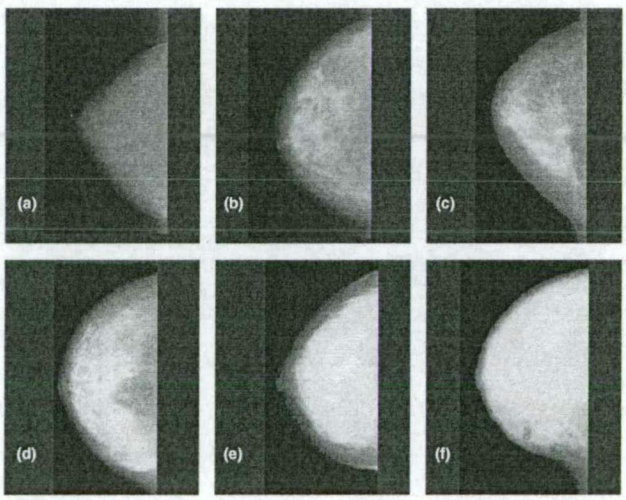
Treated girls might differ from untreated girls in some growth parameters associated with mammographic density (e.g. birthweight, birth-length, or childhood height). From previous reports it is known that untreated girls generally have less growth potential than treated girls, reflected by a greater level of pubertal maturity and more advanced bone age when compared to their chronological age[77]. Finally, a comparison of studies of association

between a number of childhood/ adolescent growth parameters and mammographic density is undertaken.

6.1 Mammographic density – what is it?

Fat is radiolucent and appears dark on a mammogram, while stromal and epithelial tissue appears light[4]. The area of tissue that appears light on a mammogram is referred to as breast or mammographic dense tissue. **Figure 6.1** shows representative images of mammograms of varying degrees of density from (a) no density to f) very dense.

Figure 6.1: Mammograms showing dense (white) and non-dense (dark) areas of the breast across varying degrees of density: a) no density to f) very dense.



Example for each of the categories for classifying mammographic density using the six category system. Fibroglandular tissue is categorised as follows: **(a)** 0, **(b)** <10%, **(c)** 10-25%, **(d)** 26-50%, **(e)** 51-75%, **(f)** >75%. Source[2] which was a clearer reproduction from[4]

6.2 How is mammographic density measured?

Mammographic density can be measured using qualitative descriptors or quantitatively by visual estimation or with computer assistance. Since the studies reviewed later in this chapter use different methods of mammographic density measurements, each of these measurement methods are described below.

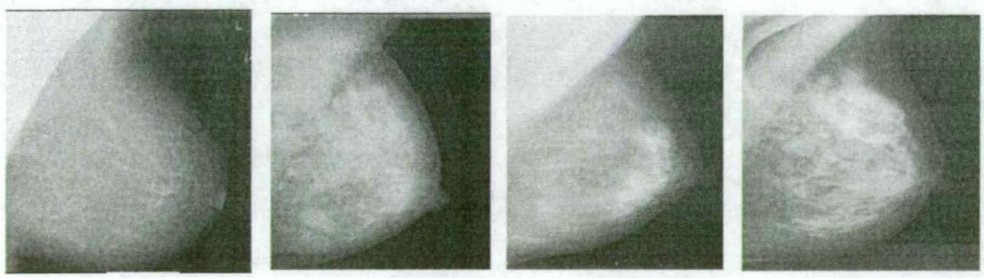
6.2.1 Qualitative measurement of mammographic density

Qualitative methods of mammographic measurement include the Wolfe grading system, and the BI-RADS scaling system. These two qualitative systems use four categories of density classification, and consequently, are only able to detect large changes or differences in density[272]. They are measured by visual estimation and are more subjective than the quantitative measures of density. The Wolfe and BI-RADS systems of measurement are each described below.

6.2.1.1 Wolfe grade

John Wolfe first defined four categories of breast parenchyma breast patterns that could be used as an index of risk for developing breast cancer in 1976[273]. It is a subjective scale that takes into account the quantity of density and nature of the density in the breast. The categories and their representation are as follows: N pattern represents a fatty radiolucent breast, P1 and P2 patterns refer to greater levels of prominence of fibroglandular tissue (hence density) while the DY pattern refers to dense sheets of fibroglandular tissue. See **Figure. 6.2** for examples of mammograms that represent these four categories.

Figure 6.2: Representative mammograms of each of the Wolfe Grades of mammographic density measurement. Source Ruth Warren.



N1 P1 P2 DY

6.2.1.2 (BI-RADS™)

BI-RADS™ refers to the Breast Imaging Reporting and Data Systems of categorising breast density developed by the American College of Radiology[274]. The four categories are as follows: 1: entirely fat, 2: scattered fibroglandular densities, 3: heterogeneously dense and 4: extremely dense.

The BI-RADS mammographic density categories have been matched to quantitative quartile measures of percent density: <25% dense for almost entirely fatty, 25–50% dense for scattered fibroglandular densities, 51–75% for heterogeneously dense, and >75% dense for the extremely dense category. The accuracy of the new definitions was evaluated by Nicholson and colleagues[275]. They found that fatty and extremely dense BI-RADS categories compared relatively well to the percent density definitions but the scattered fibroglandular densities and heterogeneously dense categories (the middle categories) did not perform as well[275]. This discrepancy has also been observed elswhere[276] and is consistent with the explanation for the poor inter-observer reliability estimates reported below[277].

6.2.2 Quantitative methods

Mammographic density can also be measured quantitatively as percent or dense area, and estimated visually with or without computer assistance. Examples are described below.

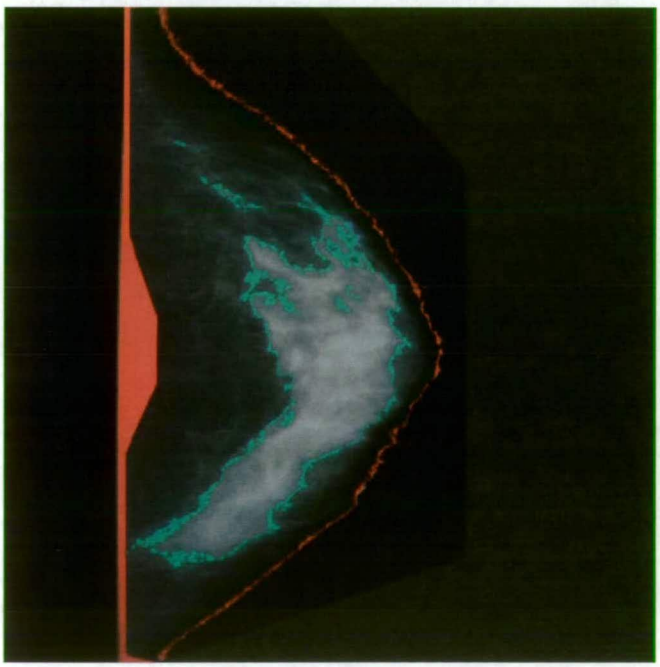
6.2.2.1 Visual estimation

Percent density has been visually estimated on a continuous scale[278] or using a six category classification scheme developed by Boyd and co-workers[4]. **Figure 6.1** above contains representative images of each of the six classifications of percent density: (a) 0, (b) <10%, (c) 10–25%, (d) 26–50%, (e) 51–75%, (f) >75%.

6.2.2.2 Computer assisted method of measurement

Computer assisted methods of measurement are believed to be less subjective than the visual estimation methods described above. This method requires the mammogram to be scanned and digitised for viewing on the computer screen. Using an interactive computer method[279] with software such as Cumulus[280], the reader chooses a brightness level defining dense tissue, and defines the boundary of the breast outline, and separately, the boundary of breast dense tissue. The computer program calculates the number of pixels in the digitised image of the breast within the margins determined by the operator for total breast area and dense area (the area that shows up white on the mammogram). (See **Figure. 6.3** for an illustration of these boundaries). The computer program then calculates non-dense area in pixels by subtracting dense area from total breast area, and percent density which is the proportion of the breast area that is dense.

Figure 6.3: Image as it appears on the screen with the breast area (red) and dense area (green) outlined.



These images are two-dimensional (2D). Volumetric three-dimensional (3D) methods such as MRI and ultrasound tomography[281], are available but are still being validated. MRI has been recently used on young women by Lisa Martin and Norman Boyd in Canada[282]. Wei et al. (2004)[283] examined the correlation between percent density measurements using the 3D MRI method and a 2D method (segmented approach). The two measures were highly correlated (0.91), while Boyd et al. (2009)[282] found percent water, as measured by MRI, to be strongly correlated with percent mammographic density (r=0·85).

Some degree of subjectivity is still involved in the 2D computer assisted method. The computer assisted methods also require specific software and density measurement training. For this reason, according to Duffy et al. (2008)[278], some large studies still prefer visually assessing percent breast density.

6.2.2.3 Planimetry

The 2D measurement of percent or absolute density can also be preformed directly using a planiometer. Planimetry involves tracing the edges of dense tissue on the mammogram and the total area (2D) of the breast. From these measurements percent density can be calculated. The reader is able to draw boundaries at different levels of brightness across different areas of the film, thereby allowing for different compressions across the breast. The computer assisted thresholding technique does not provide the option to alter the brightness across different parts of the breast. While less subjective than the visual estimation methods, the planimetry method has been reported to be labour intensive and not practical for large numbers of mammograms[2].

6.2.2.4 Reliability assessments of the different methods of measurement

A recently published paper by Gao et al. (2008)[284] described the comparative inter-rater and intra-rater reliability assessment of qualitative and quantitative visual subjective mammographic density methods of measurements. The inter- and intra-rater reliability scores for the Wolfe grade system of measurement were: weighted kappa 0.89 ($p<0.0001$) and 0.87 ($p<0.0001$), respectively[284]. These reliability estimates were similar to those for the six category quantitative method of measurement of percent density described below at 0.84

($p<0.0001$) and 0.86 ($p<0.0001$) respectively[284]. The inter- and intra-rater agreement for the visual estimation of percent density on a continuous scale has been reported to be higher again: intraclass correlation coefficient (ICC) 0.94; and 0.96 respectively[284].

Three studies calculated the kappa values for intra-observer agreement for the BI-RADs method of measurement. The values were 0.77 (95% CI: 0.69 to 0.85)[285], 0.72 (range 0.66 to 0.78)[286], and 0.71 (range 0.32 to 0.88)[277]. Inter-observer agreement was much lower: kappa 0.59 (range 0.55–0.62)[286] and 0.54 (range 0.02–0.77)[277]. For one of the studies, the low level of inter-observer agreement was partly due to a major disagreement in the intermediate categories[277].

McCormack et al. (2007)[287] examined the reliability of a volumetric method and the more common 2D thresholding method described above. They 2D method was found to have a higher reliability score (ICC 0.92) compared to the 3D method (0.77).

While the continuous quantitative measurement of mammographic density (as percent or absolute density) produces the highest reliability scores, it is also provides a more sensitive measure of change in density compared with the qualitative measures and categorical quantitative measures described above. The broader qualitative categories or the 6-category measure of percent density can only detect changes that are large enough to warrant a change in density category. For this reason, longitudinal studies of change in mammographic density should aim to use a continuous measure of density.

6.3 Mammographic density and breast cancer risk

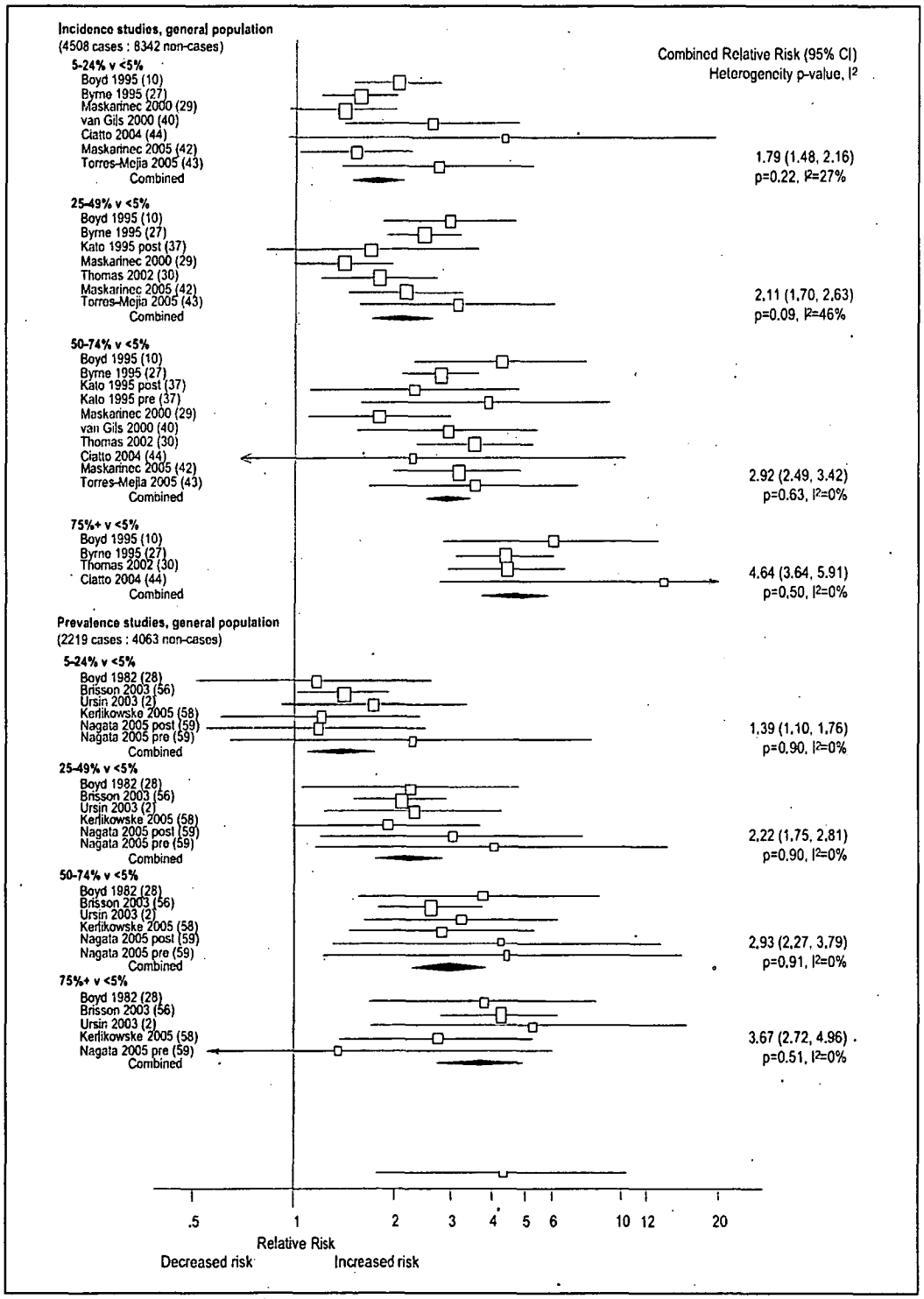
Mammographic density is an outcome of interest in this PhD investigation because of its association with breast cancer risk. Mammographic density has been consistently found to be strongly and positively associated with risk of breast cancer. Boyd and colleagues[4] undertook a review of studies that examined the association between mammographic density and breast cancer. Of the 34 studies that measured density qualitatively, and nine case-control studies that measured percent mammographic density quantitatively, all observed an association between mammographic density and breast cancer. A meta-analysis of the quantitative

studies showed that women with dense tissue in more than 60–75% of the breast had 4–6 times greater risk of breast cancer than those with a lower proportion of dense tissue after adjustment for relevant covariates[4]. Boyd et al. (1995)[36] previously estimated that, on a continuous scale, a 1% increase in percent breast density (multivariable adjusted) equated to a 2% increase in the relative risk (RR) of mammographic cancer and a 406 mm^2 (4.06 cm^2) change in total dense area equated to a 3% increase in RR[36].

A more recent review published in 2006 by McCormack and dos Santos Silva[288] involved a meta-analysis of 42 published studies that had examined the association between breast cancer risk and mammographic density. The reviewers examined the possible reasons for disparity between studies and observed a difference between incident and prevalent breast cancer studies. They combined the relative risks of incident breast cancer across five categories of percent mammographic density using findings from nine nested case-control or cohort studies (4,508 cases, 8,342 controls). The combined relative risks for incident breast cancer were 1.79 (95% CI: 1.48 to 2.16), 2.11 (95% CI: 1.70 to 2.63), 2.92 (95% CI: 2.49 to 3.42), and 4.64 (95% CI: 3.64 to 5.91) for percent mammographic density categories 5 to 24%, 25 to 49%, 50 to 74%, and >75% relative to <5%. They also combined the relative risks of prevalent breast cancer derived from five case-control or cross-sectional studies. While slightly lower than the relative risks observed for incident breast cancer, a positive trend was evident; RR 1.39 (95% CI: 1.10 to 1.76), 2.22 (95% CI: 1.75 to 2.81), 2.93 (95% CI: 2.27 to 3.79) and 3.67 (95% CI: 2.72 to 3.79) for percent mammographic density categories 5 to 24%, 25 to 49%, 50 to 74%, and >75%[288] respectively (See **Figure 6.4**). Breast cancer risk was 4–5 fold larger in women with >75% dense breasts compared with women with less than 5% dense breasts[288]; consistent with Boyd's earlier estimates[4].

The majority of research studies on mammographic density have used the relative measure of density (% density). The studies that also used the absolute measure of dense area have also observed a positive association with breast cancer risk[289-293]. In a comparative assessment, Torres-Maija and co-investigators found absolute density to be a stronger predictor of breast cancer risk than percent mammographic density[290]. This has been supported by others[289, 294] but not all[292, 295, 296]. Mostly, it appears that the two measures are similar in their association with breast cancer risk[292, 295, 296].

Figure 6.4 Study specific and combined relative risks of breast cancer (incidence and prevalence) with increasing percent mammographic density. Sourced from McCormack and dos Santos Silva[288].



The strength of the evidence supporting mammographic density as an important breast cancer risk factor presented above has led to suggestions that it be included in breast cancer risk prediction models[297-301].

6.4 Hormone exposures and mammographic density

It is unknown whether that treatment with high-dose estrogens in adolescence affects mammographic density later in life. No studies have examined this possible association, however, a number of studies have examined the effect of exogenous and endogenous hormone levels on mammographic density at other life stages[§§§]. These are summarised below.

6.4.1 Exogenous hormones and mammographic density

It has been shown that exogenous hormones influence mammographic density. HRT (both E and E&P combined formulations), tamoxifen, and gonadotropin releasing hormone agonist (GnRHa) are some examples. HRT increases mammographic density, while tamoxifen, regarded as an estrogen antagonist, and GnRHa which blocks ovarian function, reduce mammographic density. A review of the studies that have investigated these associations is presented below.

[§§§] Studies were identified by a PubMed search of the English language literature using the terms mammographic (OR breast AND density OR parenchymal patterns) AND hormone OR o/estrogen OR estradiol OR contraceptive OR progestagen/progesterone OR IGF-I or insulin) for any field covering all dates up to time of writing. The reference lists of all the publications identified by this search were inspected for additional studies. The findings and characteristics of all studies and/or reviews of studies that had explored and reported the association between hormones and mammographic density are described. When an attempt to describe all studies for an association excluded a study, the study and the reason for exclusion is described in the footnote of the relevant table.

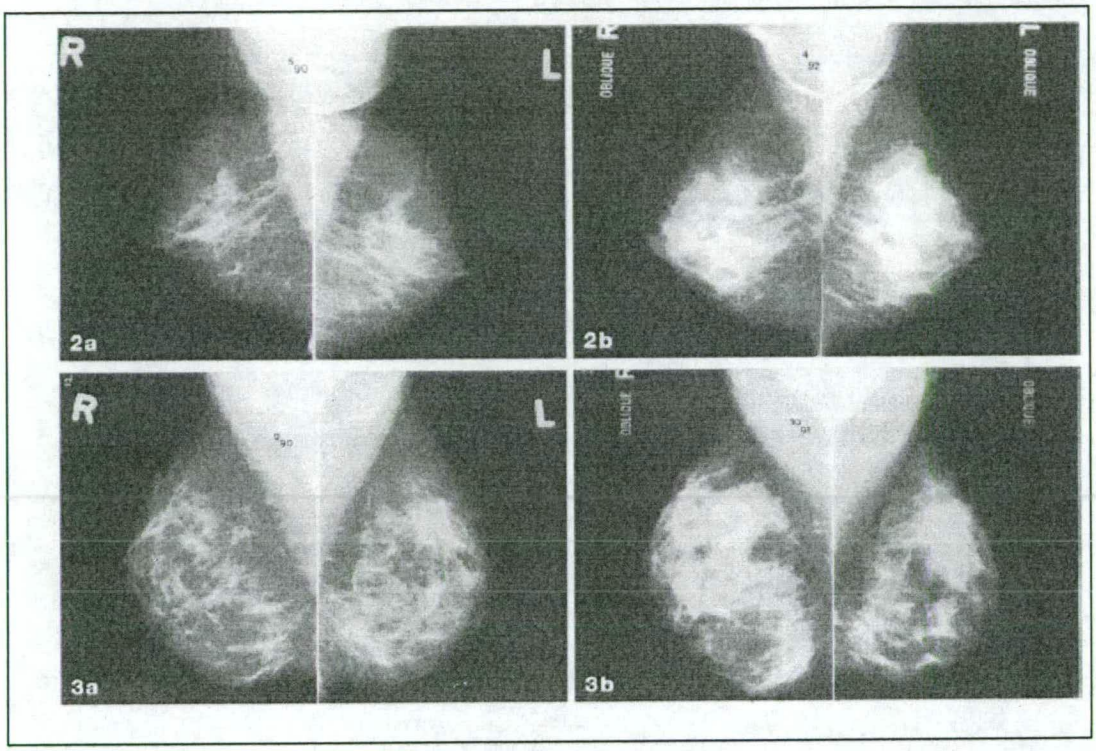
6.4.1.1 Hormone replacement therapy and mammographic density

A large number of cross-sectional[302-306] and longitudinal[40, 243, 307-322] studies have reported increased levels of mammographic density with hormone replacement therapy (HRT) use. The studies measured mammographic density qualitatively[37, 243, 302, 303, 307, 309, 310, 312-315, 317-323] or quantitatively, as percent density[304, 305, 309, 312, 316, 317] or dense area[304, 324]. The quantitative studies classified mammographic density in categories[309, 311, 312] (e.g. <25%, 25–75%, and >75%)[319] or used the continuous measure (e.g. % density, or cm^2)[305]. The different study types are reviewed separately beginning with cross-sectional studies.

Cross-sectional studies: continuous measures of mammographic density

It is not possible to measure change in mammographic density using the cross-sectional study. Change can be measured in longitudinal studies. See **Figure 6.5** for an illustration of changes in mammographic density in two women using HRT from baseline. While the cross-sectional design cannot measure change in density directly, it can be used to measure differences in mammographic density between groups (e.g. between users and non-users of HRT), or to measure associations (e.g. between mammographic density and duration of HRT use).

Figure 6.5: Baseline and follow-up mammograms after 13-24 months of HRT use in two different women (2a & b and 3a and b). Sourced from Marugg et al. (1997)[321].



Four of six cross-sectional studies that used continuous measures of mammographic density are summarised in **Table 6.1.** Of these studies, current users of HRT had between 3.3 to 7.0% greater percent density compared with never or former users[304-306, 325]. The cross-sectional study by Bremnes et al. (2007)[304] also examined dense area. They found current users of HRT had ~9 cm^2 higher density compared with never or former users.

Two studies were not provided in **Table 6.1** because of a lack of relevant data. In their comparative cross-sectional analysis of two twin studies to determine the proportion of the residual variation in percent mammographic density explained by genetic factors, Boyd and colleagues[326] found no evidence of an independent association between percent density and current or past use of HRT (data not presented). While Martin et al. (2000)[327] undertook a study of 425 participants in the Women's Health Initiative study to identify predictors of percent mammographic density in postmenopausal women controlling for confounders. They found that previous use of HRT predicted a lower percent mammographic density (data not shown), but only among women who had had a hysterectomy.

Cross-sectional studies: qualitative or categorical measures of mammographic density

Seven cross-sectional studies that used qualitative measures of density are summarised in **Table 6.2.** Of these, five observed a greater proportion of HRT users with a higher density category compared to never or former users. El-Bastwissa et al. (2000)[328] observed an age effect on the association between HRT use and higher grades of mammographic density. The odds ratio for women who currently used HRT compared to never used was 1.4 (95% CI: 1.2 to 1.7), 1.8 (95% CI: 1.5 to 2.0) and 2.2 (95% CI: 2.0 to 2.5), for women aged 45–55, 56–65, and >65 years, respectively. This difference between non-users and users appears to widen with age. Kaufman et al. (1991)[303] observed a higher proportion of low density Wolfe patterns with ageing in non-HRT users compared to users. This further supports the suggestion made by Maskarinec et al. (2006)[329], and Sterns and Zee[311] that HRT inhibits involutional processes within the breast resulting in an higher dense patterns with age.

Table 6.2 Cross-sectional studies of association between HRT and mammographic density measured qualitatively.

Study	Country	N	Age*	Density Measure	Results	Adjusted
El-Bastawissi et al. (2000)[328]	USA	17,978	20–79	BI-RADS	OR 1.2 (95% CI: 1.1 to 1.3) former vs never used OR 1.8 (95% CI: 1.7 to 1.9) current vs never used of having highest two grades of density	Age, parity, age at first birth, and BMI
Kaufman et al. (1991)[303]	UK	595	54	Wolfe grade	No significant difference between low and high risk patterns and duration of HRT use (p=0.74) Higher proportion of low risk patterns with ageing was observed in non-HRT users compared with women who used HRT for 5 or more years	–
Roubidoux et al. (2003)[306]	USA	528		BI-RADS	Current users are more likely to have a higher BI-RADS category than non-users of HRT (p=0.05)	Age, parity, ethnicity
Roubidoux et al. (2003)[323]	USA	455	53	BI-RADS	Current estrogen HRT users were more likely to have a higher BI-RADS category than non-users (p=0.03)	Age, weight, parity
Sala et al. (2000)[302]	UK	400	NS	Wolfe grade	OR 2.48 (95% CI: 1.32 to 1.61) current vs non-users of having a higher BI-RADS category	Menopause, parity, BMI, history of benign breast disease
Bland (1980)[330]	USA	129	60	Wolfe grade	No increase in the incidence of the highest Wolfe Grade in users vs non-users (p=0.168)	NS
Bergkvist et al. (1989)[331]	Sweden	35,560	>35	Wolfe grade	OR 1.36 (95% CI: 1.24 to 1.49) for user during 3 year period vs non-user during 3 year period, of having higher Wolfe grade	Age, parity, age at first parity, history of breast cancer and biopsy, family history of breast cancer

NS: Not specified * If no range specified, age is stated as the mean.

Excluded study by Leung et al. (1997)[332]. Scoring system for density grade not clearly defined. Also excluded study by Panpanit et al. (2004)[333] who had examined the effect of HRT on mammographic density change in 66 hysterectomised women, and observed an increase in density in 2 women after 12 months of HRT use using BI-RADs. Baseline for some women (number unknown) began 3 months into use of HRT.

Table 6.1: Cross-sectional studies of the association between HRT and percent mammographic density and dense area measured quantitatively.

Study	Country	N	HRT type	Effect Size	Adjusted
Percent Mammographic Density					
Vachon et al. (2000)[305]	US	1554	HRT	+ 5% in current vs never/former users (p=0.001)	Age, BMI, WHR, family history of breast cancer, physical activity, education, alcohol, age at menarche, age at first birth and number of births, smoking history
Gapstur et al. (2003)[325]	USA	296	HRT	+ 3.3% in current vs never/former users (p=0.03)	Age, BMI, and parity
Bremnes et al. (2007)[304]	Norway	1007	All	+ 3.6% in current vs never users (P for trend<0.001)	Age, BMI, parity
			E + P*	+ 6.1% in current vs never	
				+ 4.8% in <5 years use vs never	
				+ 7.0% in ≥5 years use vs never	
Roubidoux et al. (2003)[306]	US	528	HRT	+ 4.6% in current vs never users (p=0.001)	Age, parity, history of biopsy
Dense Area cm^2					
Bremnes et al. (2007)[304]	Norway	1007	E + P*	+8.7 cm^2 <5 years use vs never (P for trend<0.001)	Age, BMI, parity
				+10.9 cm^2 ≥5 years use vs never	

*Progestagen is continuously administered. Study by Boyd et al. (2002)[326] is not included in the table because relevant data is not provided. Findings are described in text.

Longitudinal studies: continuous measures of mammographic density

Unlike cross-sectional studies, longitudinal studies can measure change in mammographic density following HRT use. Longitudinal studies using continuous quantitative measures and qualitative measures are reviewed separately in this section.

Longitudinal studies using continuous measures of mammographic density are summarised in **Table 6.3**. Three of the five RCTs described in the table had a 12 month follow-up. Of these, Descensi et al. (2004)[334] found no significant difference between the treatment (E + P) and placebo groups after 12 months, while the larger RCTs by Greendale et al. (2003)[316] and McTiernan et al. (2005)[335] observed mean percent mammographic density increases of 5.0%[334] and 8.8%[335], respectively. If these increases in percent mammographic density were sustained, they would equate to a 10–17% increase in the relative risk (RR) of breast cancer according to Boyd's[36] risk estimation model reported above (Section 6.3).

The RCTs by Lundstrom et al. (2007)[336] and Freedman et al. (2001)[337] followed up women for six months and 24 months, respectively. Freedman observed a significant difference in the 24 month change in percent mammographic density between placebo and the group of women who had received postmenopausal estrogen therapy. This is in contrast to the 12-month follow-up in the PEPI study by Greendale et al. (2003)[316] reported above, which found no effect with estrogen only therapy. It is possible that a longer follow-up of 24 months might be needed to observe an effect with estrogen only formulations.

Lundstrom observed no significant 6-month change in percent mammographic density between placebo and the group who received estrogen and progestagen combined HRT. Six months follow-up might have been too short to observe a significant effect. Though Decensi et al. (2004)[334] also found no effect with E and P combined HRT and their study involved a longer follow-up of 12 months. Also, the two studies that did observe a significant increase in mammographic density following estrogen and progestagen combined formulation HRT compared to placebo (Greendale et al., 2003 and McTiernan et al., 2005) had larger sample sizes than the studies that did not find a significant change following treatment.

Table 6.3: Longitudinal studies of the association between HRT and percent mammographic density (PMD) measured quantitatively*.

Study	Country	Follow-up	N	Treatment type	Effect Size (Change in PMD)
Greendale et al. (2003)[316]	US	12 months (RCT)	571	Placebo	–0.05% (95% CI: –1.50 to 1.38)
				E	+1.3% (95% CI: –0.05 to 2.63)‡
				E + cyclic P	+5.1% (95% CI: 3.5 to 6.7)
				E + continuous P	+4.5% (95% CI: 2.7 to 6.3)
Decensi et al. (2004)[334]	Italy	12 months (RCT)	227	Placebo	+4.1% (95% CI: 2.5 to 5.6)
				E + cyclic P (oral)	+3.0% (95% CI: 1.5 to 4.4) ‡
				E + cyclic P (transdermal)	+4.0% (95% CI: 2.5 to 5.5) ‡
McTiernan et al. (2005)[335]	US	12 months § (RCT)	413	Placebo	–1.1% (95% CI: 0.3 to 1.9)
				E + continuous P	+7.7% (95% CI: 5.9 to 9.5)
Lundstrom et al. (2007)[336]	Sweden	6 months (RCT)	255	Placebo	+0.7%
				E + continuous P low-dose 1	–0.2%‡
				E + continuous P low-dose 2	+1.4%‡
Freedman et al. (2001)[337]	Multiple	24 months (RCT)	168	Placebo	–1.3% (95% CI: –2.2 to –0.4)
				E	+1.2% (95% CI: –0.6 to 3.0)†
Laya et al. (1995)[317]	US	12 months	41	E + continuous P	+6.7% (95% CI: 2.5 to 11.0)
Eiletsen et al. (2008)[338]	Norway	12 months	202	E + P (low-dose)	+2.6% ¶ (p<0.0001)
				E + P (standard dose)	+2.3% ¶ (p<0.0001)
Van Duijnhoven et al. (2007)[324]	Netherlands/UK	3 years (mean)	1240	Placebo	–7.4%
				E + P	–3.4% (p<0.01 cf. placebo)

* All results above unadjusted. Age and BMI similar between comparison groups. Characteristics not reported by Laya. et al. (1995)[317]. Age was not associated with change in mammographic density. Greendale et al. (2003)[316] also adjusted for baseline percent density, age, BMI, alcohol use, smoking, level of physical activity, 12-month change in BMI, clinic site, and hysterectomy status; but results similar to unadjusted. All treatment arms significantly different from placebo.

† Not significantly different to baseline, but statistically significantly different to placebo.

‡ Not statistically significantly different to placebo.

§ 24 months also reported, but not in table. (results above only provided for those women who were adherent to treatment (or non-treatment).

¶ Volumetric method of measurement, compared to baseline.

Excluded cross-sectional paper by Martin,C. et al. (2000)[327] conference abstract only. Insufficient information. Excluded Ursin et al. (2004)[339], sub-group analysis of same PEPI cohort (n=452) described by Greendale et al. (2003)[316], results the same.

Of the remaining three studies, Laya et al. (1995)[317] and Eiletsen et al. (2008)[338] did not use a placebo group. They compared baseline and 12-month percent mammographic densities following estrogen and progestagen combined HRT use. Twelve-month changes in percent mammographic densities were 6.7% and 2.3% respectively. These differences were statistically significant. It is possible that these changes might have occurred without treatment. However, since percent mammographic density reduced in three of the five placebo controlled studies, it is likely that HRT contributed to the positive changes observed in the studies by Laya et al. (1995)[317] and Eiletsen et al. (2008)[338]. The study by Eiletsen et al. (2008)[338] was the only to use the volumetric method of mammographic measurement and observed a similar increase in percent volume density for both low dose and standard dose combined HRT compared to baseline.

Unlike the other studies in **Table 6.3** , the retrospective longitudinal study by Van Duijnhoven et al. (2007)[324] observed a reduction in mammographic density with HRT use with age. These investigators observed an age related decrease in mammographic density in (E & P) HRT users (–3.4%) over the study period (multivariable adjusted), but this reduction was less than the reduction observed in women who had never used HRT (–7.4%, $p<0.01$). Similarly, for dense area, the mean reduction was –9.4 cm^2 in the placebo group and –5.6 cm^2 in the HRT user group ($p<0.01$ for difference). The investigators explained this result by suggesting that HRT reduced the age related reduction in mammographic density[324]. It is unclear why this study observed a decrease in mammographic density with HRT use, while other longitudinal studies observed an increase in mammographic density. This difference could be explained by the length of follow-up. The longitudinal studies described in **Table 6.3** had a follow-up of between 6–24 months while the median follow-up in Van Duijnhoven's study was three years. Another possible explanation is the ages of the cohort since the age-related reduction in mammographic density has been observed to be modified by age as described above and elsewhere[329, 335]. However, age does not appear to be an issue here. The mean age of the cohort in the study by Van Duijnhoven et al. (2007)[324] was 55 years and is similar to most of the longitudinal studies described in **Table 6.3**. Both studies by Laya et al. (1995)[317] and Greendale et al. (2003)[316] had a mean cohort age of 56 years. The mean age of the cohorts in the studies by Freedman et al. (2001)[337] and McTiernan et al. (2005)[335] were 52 years and 62 years, respectively.

Longitudinal studies: qualitative or categorical measures of density

The longitudinal studies reported above measured percent density or dense area as a continuous variable. A larger number of longitudinal studies used categorical or qualitative measures of mammographic density. These are summarised in **Table 6.4**. Each of these studies reported the proportion of women who had experienced a change in mammographic density with HRT use. Of the studies that examined the effect of estrogen only HRT on mammographic density change, between 0% (Berkowitz et al.,1990[340]) and 22% (Erel et al., 2001[320]) of HRT users had experienced an increase in mammographic density. The study by Berkowitz et al., was the only study where none of the women exposed to estrogen HRT experienced an increase in mammographic density, however, this study had a number of limitations that include a small sample size (n=14), and a varied exposure duration (1–72 months) and follow-up period (3–14 months). However, in this same study, 17% of women who had used estrogen and progestagen combined HRT had experienced an increase in density.

Only 14 of the 25 studies described in **Table 6.4** included a control or placebo group. Those without cannot exclude the possibility that the change in mammographic density observed after estrogen HRT might have occurred anyway. Of the studies that did include a control group and examined the effect of estrogen only HRT on mammographic density, five presented a test of significance or confidence intervals. Each of the studies by Colarcurci et al. (2001)[319], Christodoulakos et al. (2003)[341] and Orguc et al. (2006)[342] observed a statistically significant increase in the number of women whose mammographic density increased after estrogen HRT, compared to a control or placebo group; $p<0.05$, 0.02 and <0.05 respectively. Greendale et al. (1999) observed an increase in mammographic density in 3.5% (95% CI: 1 to 12) of women who had used estrogen only HRT for 12 months.

Sterns and Zee[311] followed up women over menopause and not only reported the proportion of HRT users and non-users whose breast density increased, but also the proportion whose density reduced and remained the same over the duration of the study period (from approximately 46 to 55 years of age) (See summary in **Table 6.4**). Of 117 nonusers of HRT followed over menopause, 38% had a density decrease to a lower category

before age 55, 62% had no change, while none showed an increase in density. In comparison, the density decreased in 18%, remained stable in 80% and increased in 2% of HRT users[311] (p-values or confidence intervals were not provided). The investigators suggested that HRT preserved the existing dense tissue in the majority of women by negating the age-related decline observed in non-HRT users[311], similar to that observed by Maskarinec et al. (2006)[329] who measured percent mammographic densities from mammograms collected consecutively over a 20 year period. In a shorter follow-up of women using HRT (mean 27 months), Sterns and Zee observed an increase in percent mammographic density (from one category to the next) in 8% of women following HRT use[311].

It becomes clear from the studies summarised in **Table 6.4** that mammographic density does not increase in all women exposed to HRT. The most reported was 68% by Connor et al.[312] for continuous estrogen and progestin combined HRT. Continuous measures of density change would provide a more accurate measure of the proportion of women on HRT who experience changes in mammographic density.

Longitudinal studies: continuous measures of density (% women whose density changed)

A number of the longitudinal studies using continuous measures of mammographic density described in **Table 6.3** above also presented the proportion of women whose mammographic density increased with HRT use. In the study by Freedman et al. (2001)[337], only 30% of women who undertook estrogen replacement therapy experienced an increase in percent density greater than 1 SD above the mean placebo change. McTiernan et al. (2005)[335], on the other hand, reported 74%[335]. Laya et al. (1995)[317] observed a similar result (73% after a 12 month period). Laya and co-investigators also measured density change according to Wolfe grade. Using this grading system, a record of change in density was only made if an increase occurred from one Wolfe grade category to the next. Only 24% of the women experienced an increase from one Wolfe grade to another. This lower figure is similar to the proportions in the studies using categorical or qualitative measures of mammographic density described in **Table 6.4** [37, 307, 309, 320, 321]. The qualitative or categorical measures of density might not be sensitive enough to identify increases within the density categories. Counter to this argument, Sendag et al. (2001)[309] reasoned that the 73% figure

provided by Laya et al. (1995)[317], was flawed because for 41% of women in the study, the magnitude of the density change was <10%, apparently too small be visually measured. While Laya et al. measured percent density using a planimetry computer program, which is less subjective than the visual estimation of percent density, there is some merit in the statement by Sendag and colleagues. Many of the studies using categorical or qualitative measures of mammographic density could not detect changes less than 10%. Laya's finding of 73% would reduce to 32% if changes less than 10% (41% of reported changes) were not included. The result would be similar to the proportions observed for combined formulation HRT in the studies that used qualitative measures of change (**Table 6.4**).

Table 6.4 Longitudinal studies of the association between HRT and percent mammographic density measured qualitatively.

Study	Country	Study Design (follow-up time)	Density measure	Age	HRT Type (n)	Change in density (% women)	Δ
Lundstrom et al. (2001) [318]	Sweden	Longitudinal (Not specified)	Wolfe Grade*	52	E (51) (Estriol)	6%	+
				49	E (55) (Estradiol patch)	2%	+
				53	E + P continuous (52) (CEE MPA)	40%	+
Persson et al. (1997)[343]	Sweden	Longitudinal (18 months)	Wolfe Grade and visual estimation of change.	48-58	Control (554)	3%	+
					E	5%	+
					E + cyclical P	10%	+
					E + continuous P	28%	+
Stomper et al. (1990)[307]	US	Longitudinal (18 months)	Anatomically defined†	52	E (12)	17%	+
					E & P (38)	26%	+
Ozdemir et al. (1999)[344]	Turkey	Longitudinal (17–21 months)	Change recorded if >15% change	50	Control (30)	0%	+
					E (22)	18%	+
					E & cyclic P (24)	46%	+
Lundstrom et al. (1999)[315]	Sweden	Longitudinal (12 months)	Wolfe Grade*	48–51	E (50)	18%	+
					E & P continuous (50) (E2 plus NETA)	52%	+
					E & P cyclic (75)	13%	+
Christodoulakos et al. (2003)[341]	Greece	(12 months)	Wolfe Grade	~52	Control (27)	0%	+
					E (25) (CEE)	8%	+
					E + P (34) (CEE plus MPA)	11.8%	+
					E + P (35) (E2 plus NETA)	31.4%	+
Marugg et al. (1997)[321]	Netherlands	Longitudinal (1–2 years)	Wolfe Grade	50–70	Control (575)	0%	+
					E (23)	8.7%	+
					E & P (58)	31%	+

Study	Country	Study Design (follow-up time)	Density measure	Age	HRT Type (n)	Change in density (% women)	Δ
Greendale et al. (1999)[37]	US	Longitudinal (12 months) RCT‡	BI-RADS	59	Placebo	0%	+
					E (CEE)	3.5%	+
					E & cyclic P (CEE plus MPA)	23.5%	+
						16.4%	+
					(CEE plus micronised P)		
					E & continuous P (CEE plus MPA)	19.4%	+
Bergvist et al. (1989)[331]	Sweden	Longitudinal	Wolfe Grade	>35	Control (653) (age-matched)	7.0%	+
						21.7%	−
					HRT (653)	3.9%	+
						24.9%	−
Berkowitz et al. (1990)[340]	US	Longitudinal (3–14months)	Change recorded if >10% change	NS	E (14)	0%	+
					E & P (15 §)	17%	+
Erel et al. (2001)[320]	Turkey	Longitudinal (4 yrs)	Wolfe Grade	49–51	E (23) (CEE)	22%	+
					E & continuous P (26) (CEE and MPA)	35%	+
					E & cyclic P (21) (CEE and MPA)	19%	+
Sendag et al. (2001)[309]	Turkey	Longitudinal (mean 17.4 months)	Wolfe Grade and change recorded if >10% change.	50–53	E (76) (patch or oral)	3.9%	+
					E + continuous P (61) (E2/NETA) (CEE/MPA)	31.1%	+
					E * cyclic P (44)	2.2%	+
Junkermann et al. (2005)[310]	Germany & Austria	Longitudinal (RCT) (9 months)	Visual estimation of change	55	E & continuous P (159) (E2/NETA)	32.4%	+
						3.4%	−
					E & cyclic P (132) (CEE/ medrogestone)	31.2%	+
						4.0%	−

Study	Country	Study Design (follow-up time)	Density measure	Age	HRT Type (n)	Change in density (% women)	Δ
Sterns & Zee (2000)[311]	Canada	Longitudinal (27 months)	Minimal density =0; >minimal to <25% =I, >25–<75%=II, >75% =III	~46	HRT (87) (Mixed)	8%	+
Sterns & Zee (2000)[311]	Canada	Longitudinal (~9 years)	Minimal density =0; >minimal to <25% =I, >25–<75%=II, >75% =III.	46	Control (117)	0%	+
						38%	–
					HRT (45) (Mixed)	2%	+
						18%	–
Conner et al. (2004)[312]	Sweden	Longitudinal (RCT) (6 months)	Wolfe Grade & PMD visual 0–20% 21–40% 41–60% 61–80% 81–100%	55	E & continuous P (23) (Estradiol valerate/dienogest)	48% Wolfe 52% quintiles	+
				56	E & continuous P (22) (E2/NETA)	45% Wolfe 68% quintiles	+
Marchesoni et al. (2006)[313]	Italy	Longitudinal (RCT) (12 months)	Wolfe Grade	52	Placebo (16)	0%	+
					E + continuous P (35) (CEE/MPA)	45.1%	+
Chen et al. (2005)[314]	Taiwan	Longitudinal Follow-up at 1–2, 2–3, 3–4, 4–5 and >5 years	BI-RADS	50	E (200)	10% (1–2yrs) 12% (2–3 yrs) 14% (3–4 yrs) 10% (4–5yrs) 12% (>5 yrs)	+
					E + P (267)	8% (1–2yrs) 14% (2–3 yrs) 17% (3–4 yrs) 21% (4–5 yrs) 22% (>5 yrs)	+

Study	Country	Study Design (follow-up time)	Density measure	Age	HRT Type (n)	Change in density (% women)	Δ
Rutter et al. (2001)[40]	US	Longitudinal (~ 2years)	BI-RADS	67	Control (2942)	11.6%	+
						6.5%	–
				63	HRT (335)	28.4%	+
						3.3%	–
McNicolas et al. (1994)[243]	Ireland	Longitudinal (~13 months)	a)10% change	55	Control (31)	0%	+
			b) visual assessment	52	HRT (33)	a) 18% b) 27%	+
Colacurci et al. (2001)[319]	Italy	Longitudinal (12 months)	I=<25% dense	51	Control (23)	10%	+
			II=25–75%		E(23)	21%	+
			III=>75%		E + cyclic P (26) transdermal E2/acetate nomegestrolo	35%	+
					E + continuous P (25) transdermal E2/acetate nomegestrolo	43%	+
Topal et al. (2006)[345]	Turkey	Longitudinal (14 months)	Wolfe Grade	50	E (37)	2.7%	+
					E + cyclic P (16)	12.5%	+
					E + continuous P (60).	38.3%	+
Kilicdag (2004)[346]	Turkey	Longitudinal (12 months)	>10% change	50	Control (47)	0%	
					E (27)	11.1%	+
					E & P (75)	33.3%	+
Orguc (2006)[342]	Turkey	Longitudinal (16 months)	Wolfe Grade/ Segmented approach¶	49	Control (79)	0%/0%	+
					E (62)	0%/21%	+
					E + P (98)	8.1%/42%	+
Bulbul (2003)[347]	Turkey	Longitudinal (12 months)	Visual estimation of change	49	E (80)	18.8%	+
					E + cyclic P (40)	12.5%	+
					E + continuous P (44)	25.0%	+

Study	Country	Study Design (follow-up time)	Density measure	Age	HRT Type (n)	Change in density (% women)	Δ
Nahas-Neto (2006)[348]	Brazil	Longitudinal (RCT) (6 months)	BI-RADS	59 54	Control E + continuous P (E2/NETA)	16% 48%	+ +
Laya et al. (1995)[317]	US	Longitudinal (retrospective) (12 months)	Wolfe Grade	41	E + continuous P	24%	+

* Within each Wolfe category, differences of 10–25% between films were also recorded.

† No change; or change: diffuse increase in density (at least 10% change); increased multifocal asymmetric densities that did not exhibit mass effect or architectural distortion (at least 10% change); cyst development

MPA medroxyprogesterone acetate, NETA norethisterone acetate, E2 17β-estradiol

‡ Study also examined 24 and 36 months but most of the changes occurred within the first 12 months.

§ 16 patients originally but one did not have a mammogram before treatment with HRT.

¶ Novel approach called 'comparison wheel'.

Studies not included in the table: Bland et al. (1980)[330] insufficient information provided about the effect of estrogen HRT on change in mammographic density; Gerogiev 2002[349] – insufficient numbers.; Berkowitz et al. (1990)[340] unable to assess when mammograms were taken in relation to start of HRT.

Estrogen vs combined estrogen and progestin HRT.

One consistency that does exist across studies is the larger effect on mammographic density observed with combined estrogen and progestagen compared with estrogen only HRT formulations. The longitudinal studies using quantitative continuous measures (**Table 6.3**) have observed mean increases in mammographic density between 2.8% and 7.7% after estrogen and progesterone combined HRT exposure, compared with 1.2–1.3% after estrogen only HRT exposure.

In the majority of the longitudinal studies using qualitative or categorical measures of mammographic density described in **Table 6.4**, the proportion of women who experienced an increase in mammographic density following exposure to combined HRT ranged from 2 to 68%, with the majority of studies reporting between 20% and 40%. This is much larger than the proportion who experienced an increase in mammographic density following estrogen only HRT (3.9 to 22%). Statistically significant differences in these proportions, between combined and estrogen only HRT exposures were observed in the following studies: Ozdemir et al. ($p=0.04$)[344], Marugg et al. ($p=0.046$)[321], Greendale et al. ($p=0.024$)[37], Berkowitz et al. ($p=0.045$)[340], Sendag et al. ($p=0.001$)[309] and Kilicdag et al. ($p=0.04$)[346]. Statistical significance was not observed in the studies by Stomper et al. ($p>0.70$)[307], Christodoulakos et al ($p\geq0.18$)[341], and Erel et al. (p not stated)[320], though in the latter studies, the size of the samples were small (see **Table 6.4**).

Martin et al. (2000)[327] observed a lower percent mammographic density among HRT users who had had an hysterectomy and suggested that the higher frequency of oophorectomies in this group may be the cause of the lower percent density and consequently increase the likelihood of HRT use. This is an interesting point because in most studies using E only HRT, the women have had a hysterectomy. The combined formulation HRT is typically used in women who have not had a hysterectomy, and it is the E and P formulations that more frequently lead to an increase in density in treated women as observed above. It is possible that the women using estrogen only HRT are experiencing dual responses – a reduction due to oophorectomy and an increase due to the exogenous estrogen. However Greendale et al. (2003)[316], randomly assigned women to estrogen only or estrogen and progestin combined HRT and adjusted for hysterectomy status. Estrogen only HRT was

found not to have any effect on mammographic density. This does not explain, though, the increases observed in mammographic density with E only HRT in some studies.

Cyclic vs continuous combined HRT

Potentially, the type or frequency of progesterone in the HRT regimen might also determine the size of effect on mammographic density. The progestagen can be taken cyclically (a few days a month) or continuously (daily). This is of particular relevance to this PhD study because girls treated with high-dose estrogens for the treatment of tall stature were typically given a progestagen for a few days each month (cyclically). A number of studies have examined the association between mammographic density and the two forms of combined HRT (See **Tables 6.3** and **6.4**) In the Turkish longitudinal study by Sendag et al. (2001)[309], **(Table 6.4)** a greater proportion of women who were treated with continuous combined HRT (31.1%) experienced an increase in Wolfe grade from baseline (mean follow-up 28 months) compared with women who were treated with cyclic combined HRT (2%) (p=.0002). Lundstrum et al. (1999)[315] also reported a difference in the Wolfe grade increase between women treated with continuous combined formulations (52%) compared to those treated with cyclic combined HRT six months following treatment (13%, p-value or confidence intervals not provided). A difference was similarly observed by Topal et al. (2006)[345]; 38% and 12%, respectively (p<0.001). However a number of studies did not observe any statistical significant difference between the two E & P regimens, these include: the PEPI trial reported by Greendale et al. (2003)[316], and Ursin et al. (2004)[339] **(Table 6.3)** , the RCT study by Junkermann et al. (2005)[310] that used a visual estimation of change in mammographic density, and the non-randomised controlled trial by Colarcurci et al. (2001)[319] that measured increases in density according to Wolfe Grade **(Table 6.4)**. The study by Erel et al. (2001)[320] observed a greater proportion of continuous combined users (35%) than cyclical combined users (19%) showing an increase in Wolfe grade after HRT use but the difference was not statistically significant (p-value not provided). It is unclear why the more insensitive measures of change used by Lundstrom et al. (1999)[315], Sendag et al. (2001)[309] and Topal et al. (2006)[345] detected a difference in effect between cyclic and continuous forms of combined HRT while the more sensitive quantitative measure used in the PEPI trial did not.

Type of progestagen

It should be noted that progestins, like estrogens, have different potencies and physiologic actions. For instance, some have androngenic, nonadrogenic and antiandrogenic actions[350]. The type of progestagen used in the combined HRT formulations might also influence the effect observed on mammographic density. The two common forms of progestagen used in combined HRT formulations include norethisterone acetate (NETA) and medroxyprogesterone acetate (MPA). In the study by Sendag et al. (2001)[309] (**Table 6.4**) a greater proportion of women experienced an increase in percent density category following continuous combined HRT with norethisterone acetate (34.1%) compared to women using medroxyprogesterone acetate (23.5%), though this difference was not statistically significant (p=0.42). Christodoulakos et al. (2003)[341] also examined the difference between NETA and MPA on percent mammographic density, and like Sendag et al. (2001)[309] observed a more frequent increase in percent density in women using NETA (31.4%) compared with women using MPA (11.8%), but the difference was not statistically significant (p=0.3). Nor was the difference in the proportion of women who had a BI-RADs category increase following HRT containing micronised progestagen (16.4%) and MPA (23.5%) in the PEPI trial by Greendale et al. (1999)[37] statistically different (**Table 6.4**). No difference in the frequencies of increases in percent density were observed between the two progestagens MPA with cyproterone acetate[344] or NETA and dienogest[312].

Tall girls were typically treated with estrogen plus a cyclic progestagen. The evidence presented above suggests that E only formulations increase mammographic density, but for most studies, the effect was stronger with the combined E + P formulations. Similarly for combined formulations with cyclic compared to continuous progestagen. While effects of E and cyclic progestagen have been shown to affect mammographic density, the continuous progestagen formulations have been shown to have a larger effect again.

HRT induced breast pain and mammographic density

Of particular interest to the tall girls follow-up is the observation made by two studies that breast pain in relation to HRT use is associated with an increase in mammographic density.

In a prospective study of HRT users, McNicolas et al. (1994)[243] observed that seven of nine women (78%) who had experienced an increase in mammographic density after commencing HRT, had also experienced moderate or severe breast pain. In contrast, only five of 24 (21%) of those who did not experience an increase in mammographic density experienced moderate or mild breast pain. No women belonging to a control group of non-HRT users (n=31) reported breast pain during the same follow-up period.

Crandall et al. (2006)[241], observed a similar association in a subset of the PEPI RCT cohort. Women who experienced the onset of breast discomfort within a 12 month period following HRT commencement, had a 3.9% increase (age and multivariable adjusted) in mean percent mammographic density following HRT use, compared with an 0.6% increase in women who did not experience breast discomfort ($p<0.001$).

As described in Chapter 3 of this thesis, a number of women in the Australian Tall Girls cohort reported breast pain as a side effect of treatment as an adolescent. It is possible that an increase in breast pain during treatment coincided with an increase in mammographic density. Whether an increase in density during adolescence remains in adulthood is unknown. Evidence suggests that the effect of HRT on mammographic density is only transient. The following section presents a review of the evidence that concerns the permanence of the effect of HRT on mammographic density.

Duration of the HRT effect on mammographic density

As it appears from the evidence above, HRT increases or reduces the age-related decline in mammographic density in postmenopausal women. From the few studies that have followed-up mammographic density changes after the cessation of treatment, it appears that this effect is not permanent. The first apparent study to examine the effects of HRT cessation on mammographic density was undertaken in the US by Harvey, Pinkerton and Herman (1997)[351]. They followed up 47 women who had either experienced an increase in mammographic density or a new mass of dense tissue while using HRT. Of these, 75% experienced a reduction in visually assessed dense tissue approximately two weeks after stopping HRT. The investigators proposed that short-term cessation of HRT might avoid unnecessary biopsy of unusual masses, and improve mammography specificity.

Colarcurci et al. (2001)[352] undertook a controlled clinical trial (not randomised), involving 97 menopausal women in one of three treatment arms: estrogen alone (n=37) and combined formulation HRT (n=39), and no medication (n=21). A mammogram was performed at study entry and after 12 months. Mammographic density was visually assessed by planimetry and classified into three categories (<25%, 25 to 75%, >75% density). Of those treated with estrogen and combined HRT, a subset (18, 20 respectively) had suspended treatment for a mean period of 22 days prior to the second mammogram. At second mammogram, mammographic density increased from one dense category to a higher category in 21% of estrogen HRT users, 37% of combined HRT users, and 0% of non HRT users. Of the subsets of the treated group that discontinued HRT shortly before the follow-up mammogram, only six and 5% of the estrogen and combined HRT users had experienced an increase in mammographic density, respectively. The increase was significantly different for those who had continued with combined HRT ($p<0.05$), but not estrogen HRT ($p>0.05$).

A larger study by Rutter et al. (2001)[40] compared the change in mammographic density in non-HRT users (n=2,942) with HRT users who had stopped using it prior to a follow-up mammogram (n=111). The investigators found that women who stopped using HRT, (up to 25 months prior to follow-up) were more likely to demonstrate a reduction in BI-RADS category of mammographic density compared with non-users of HRT over the follow-up period, (age and BMI change adjusted RR, 1.81; 95% CI: 1.06 to 2.98). These findings suggest that mammographic density changes reverse with the discontinuation of HRT.

The findings of a more recent study by Weaver et al. (2008)[353] conflict with the three studies above. In this later study, density change in 48 women was assessed four weeks following the cessation of HRT using four different methods of mammographic density measurement: Wolfe grade, six-categorical visual scale of percent density, and two computer assisted methods. No significant change in mammographic density was observed, despite the method used ($p>0.08$). Possible reasons for the inconsistency between the studies include different study populations and duration and types of HRT used. The study population used in the study by Harvey, Pinkerton and Herman only included women who had already experienced an increase in mammographic density or a new dense mass while using HRT. This subset of women may be more responsive to the cessation of HRT compared to other women.

Different HRT durations may explain the discrepancies. Mean durations of HRT use were 57 months (range six months–14 years), 12 months, and 12 months or greater (upper limit not specified) in the studies by Weaver et al. (2008)[353], Colacurci et al. (2001)[352], and Harvey, Pinkerton and Herman (1997)[351], respectively. Duration was not reported in the study by Rutter and colleagues. Different forms of HRT were used in each of the studies by Weaver et al. (2008)[353], Rutter et al. (2001)[40] and Colacurci et al. (2001)[352], but only the latter study stratified by type of HRT. HRT type was not specified by Harvey, Pinkerton and Herman.

Former versus current HRT use

It appears from the evidence presented above, that the duration of effect on mammographic density is limited to the duration of HRT treatment. Mammographic density returns to baseline levels soon after the cessation of treatment. It would be expected then, that mammographic density would be lower in former users of HRT compared with current users if all other risk factors remained the same. Three cross-sectional studies compared mammographic density levels between former and current users of HRT. Vachon et al. (2000)[305] and Gapstur et al. (2003)[325] (**Table 6.1**), reported a 3–5% multivariable adjusted increased mean percent density in current users of HRT compared with former users, while El Bastawissi et al. (2000)[328] found current users to have an increased odds of having a higher BI-RADS category of mammographic density compared with former users:, OR 1.2 (95% CI: 1.1 to 1.3) (**Table 6.2**).

6.4.1.2 Estrogen antagonists and mammographic density

It is clear from the evidence presented above, that exogenous estrogen, particularly when combined with a progestin, increases mammographic density in some women. It would be expected then, that the inhibition of these hormones would result in a reduction in mammographic density.

Many studies have examined the effect of tamoxifen, a selective estrogen receptor modulator, on mammographic density. The metabolites of tamoxifen have a high affinity for the estrogen receptor and therefore compete with estrogen for binding sites. They act as

estrogen agonists or antagonists, depending on the site of the receptors. In the breast, tamoxifen acts as an antagonist, while in the endometrium, it acts as an agonist. Its antagonist effect on the breast is the basis of its use in the treatment of estrogen receptor positive breast cancer, and as preventive therapy in women with a high risk of breast cancer[354].

A number of studies have also examined the effect of gonadotropin releasing hormone agonist (GnHRA) on mammographic density. Like tamoxifen, GnRHA has been called an 'antiestrogen'. It is a synthetic form of gonadotropin releasing hormone that stimulates the release of the pituitary gonadotropin hormones LSH and FSH. GnHRA binds to the gonadotropin releasing hormone receptor, thereby competing with the endogenous hormone equivalent. While it is an agonist, it acts by down-regulating gonadotropin releasing hormone receptor numbers and consequently, reducing LSH and FSH release from the pituitary. Since these gonadotropins stimulate estrogen synthesis in the ovary, a concomitant reduction in estrogen production results.

A more detailed review of the studies that examined the effect of tamoxifen and GnRHA use on mammographic density is presented in Appendix 5. Overall, the findings of the controlled longitudinal studies suggest that mammographic density is reduced by tamoxifen and GnRHA. For instance, in one of these studies (Chow et al., 2000)[355] reported a 4.3% mean density reduction with tamoxifen use per year over a period of 2.5 years. While, Gram et al. 2001)[39] using the computer thresholding technique of mammographic density measurement observed consecutive reductions in percent density from baseline in women using GnRHA; 9.7% (+/–3.5%; p=0.01) and 11.4% (+/–3.5%, p=0.01) after 12 months and 24 months, respectively. No statistically significant change was observed in the control group –3.2% (+/–3.0%) (p=0.30), –2.5% (+/–2.5) (p=0.47), at 12 months and 24 months, respectively. The reduction in densities, however, was not sustained after cessation of treatment. Reductions in percent mammographic density at the levels observed by Gram et al. (2001) after 24 months of GnRHA treatment, would equate to a 23% reduction in the relative risk of breast cancer using Boyd's [36] risk estimation reported above (Section 6.3). However, this reduction in risk would not be sustained after cessation of GnRHA treatment.

6.4.1.3 Oral contraceptive use and mammographic density

Another exogenous hormone of interest is the oral contraceptive pill, particularly the studies of OC use in young women and mammographic density. Only a few studies have examined the association between the oral contraceptive pill and mammographic density specifically[356-358], others have examined the association among one of many other breast cancer risk factors[302, 305, 326, 359-361]. These are described below and summarised in **Table 6.5.**

All but the two earlier cross-sectional studies by Gravelle et al. (1980)[361] and Leinster and Whitehouse (1986)[358], found no association between oral contraceptive use and percent density or density grade. The largest of these studies, by de Stavola et al. (1990)[359], initially observed a univariable association between the two, but this disappeared after adjustment for age. Both studies had observed a higher proportion of OC users with lower risk Wolf grade patterns than non-OC users. These were not adjusted for age, however the latter of these studies stratified by menopausal status. Leinster and Whitehouse (1986)[358] examined the association between low-dose and high-dose estrogen OCs with mammographic density, and while there appeared some differences, these were not statistically significant.

Jeffreys et al. (2004)[360] examined the association between percent density (six categories) and oral contraceptive use at a young age (before 20 years). Women who used the oral contraceptive pill prior to 20 years of age did not have greater odds of having a higher percent breast density category compared to older users (P for trend 0.78). A case-study report (not included in **Table 6.5)** describes the longitudinal mammographic density changes that occurred in two women after the discontinuation of Depo-Provera, an injectable progestagen based contraception, containing depot-medroxyprogesterone acetate. Dillis and Schreiman (2003)[357] compared baseline density readings, taken when the women were treated with Depo-Provera, with a later mammogram, after the women had stopped using the contraceptive. They observed an increase in mammographic density for both women. This suggests that Depo-Provera reduced mammographic density over the term of treatment, either through its action on ovarian suppression (including estrogen suppression), and that this effect was reversed after the discontinuation of treatment. Larger studies are needed to confirm these findings.

Table 6.5: Cross-sectional studies of the association between oral contraceptive pill use and mammographic density.

Study	Country	Density Measure	N	Age*	Results	Adjusted
Vachon et al. (2000)[305]	US	% density (5% increments)	1,900	47	4% difference in percent density between never and current users (P for trend 0.45)	Age, BMI, WHR, family history of breast cancer, physical activity, education, alcohol, age at menarche, age at first birth, # births, smoking
Gram et al. (2002)[356]	Norway	Parenchymal grade I-III low risk IV-V high risk	3,218	40–56	Ever users more likely to have high risk Wolfe grade: OR 1.27, 95% CI: 1.0–1.6 vs never users	Age, BMI, menopausal status, parity, age at first birth, BMI
Boyd et al. (2002)[326]	US Australia	% density	951 twin pairs.	50–55	No association between mammographic density and present or previous use. (data not shown)	Age and possibly others – NS
Jeffreys et al. (2004)[360]	Scotland	Six category classification of % density	628	59	Use in any age group or before first birth, not associated with breast density. CIs supplied	Age, BMI, birth cohort menopausal status, HRT
Sala et al. (2000)[302]	UK	Wolfe grade P2/DY- high N1/P1- low	400	NS†	OR: 0.81 (95% CI: 0.44 to 1.47) for ever vs never users at having a high-risk mammographic pattern (p=0.49)	Age matched, adjusted for BMI, menopausal status, number of children, HRT, history of benign breast disease
de Stavola et al. (1990)[359]	UK	Wolfe grade	4,954	All	No association with Wolfe grade once multivariable adjusted	Age, parity and Quetelet's Index
Leinster and Whitehouse (1986)[358]	UK	Wolfe grade	5,319	NS	OC ever users had a greater incidence of lower risk patterns lower incidence of P2 pattern.	Stratified by menopausal status
Gravelle et al. (1980)[361]		Wolfe grade	942	>38	Lower proportion of high density (P2 and DY) grades in OC users	None

* Age reported as mean/median/range † NS: not specified

6.4.2 Exogenous hormones and breast epithelial proliferation

A number of studies have also examined the effect of exogenous hormones on the proliferative activity of breast tissue. One study found mammographic density to correlate highly with the proliferative activity of breast tissue. Harvey et al. (2008)[362] measured Ki-67, a biomarker of cellular proliferation, using non-cancerous tissue from mastectomy breast samples, and compared this measure with the mammographic density of the contralateral breast. A high correlation was observed between mammographic density and Ki-67 in both the epithelial ducts (p=0.031) and lobules (p=0.023).

Studies have examined the proliferative activity of breast epithelial tissue with HRT and tamoxifen use. Human[363, 364], organ culture[365-368] and animal studies[145, 369-371] have shown estrogen or combined HRT formulations to increase epithelial proliferative activity. In contrast, tamoxifen has been associated with reduced epithelial proliferative activity[372, 373]; and observed to directly reduce proliferation *in vitro*[368]. Some studies observed no effect of HRT on proliferative activity in normal breast tissue[374] or breast cancer cells lines[375]. A US study observed lower proliferative activity in breast cancer cells of women who used estrogen or combined HRT for 10 or more years[376]. This coincided with an observation of increased cancer survival rates in HRT users in the same study. The explanation for this observation is unclear.

Of the studies that examined the effect of oral contraceptives on the proliferative activity of mammary gland epithelial tissue, most observed a positive association[364, 377-381] while others found no such association[382-384]. The studies differed in the methodology used to measure proliferation (e.g. thymidine labelling[379, 383, 384], Ki-67 antibody[364, 377, 378]), the study population, and the type of oral contraceptive used. Controlling for potential influencing factors also varied between the studies. According to Garcia et al. (2008)[377] proliferation of normal breast tissue is influenced by the phase of the menstrual cycle, chronological age, breast age, use of hormonal formulations (especially if nulliparous), and recent parity. The study by Potten et al. (1998)[383] found no increased proliferative activity in the breast tissues of contraceptive users, when age and stage of the menstrual cycle was taken into account.

Garcia et al. (2008)[377] observed an increased level of proliferative activity in younger women (under 27 years) using oral contraceptives, and, along with Williams et al. (1991)[380] only observed an increased proliferative index during the luteal phase of the menstrual cycle. There was no difference in epithelial proliferation between users and non-users of oral contraceptives during the other stages of the menstrual cycle. While estrogen contraceptives have been associated with increased proliferative activity of breast epithelial tissue[364, 379], progesterone based oral contraceptives have been associated with a greater level of proliferative activity when compared with estrogen only contraceptives[364, 379].

6.4.3 Endogenous hormones and mammographic density

Many studies have examined the association between endogenous hormone levels and mammographic density. The findings of these studies are particularly relevant to this PhD study. The treatment of tall girls with high-dose estrogens to reduce their final adult has been shown to modify the serum levels of endogenous estrogen, IGF-I and prolactin. (See Chapter 2, Section 2.4.22). Only one known study that has examined the effect of adolescent endogenous hormone levels on mammographic density. This study by Boyd et al. (2009)[282] measured mammographic density using magnetic resonance imaging in 400 young women (ages 15–30 years) and took blood samples to measure endogenous hormone concentrations. Percent breast water content is derived from the resonance images and is a measure of the fibroglandular tissue content of the breast, and therefore percent breast density[385]. Evidence of a positive association between serum levels of estradiol and percent water in women 15–19 years of age was observed. IGF-I was not associated with percent water content in women between 15–30 years of age, while a positive association was observed with sex hormone binding globulin (SHBG), a binding protein that reduces free estradiol in the circulation and growth hormone. While this seems to be the only study examining endogenous estrogen levels and mammographic density in young females, a large number of studies have examined the relationship in adult women. A detailed review of these findings can be found in Appendix 6.

Of these studies, eight studies examined the association between circulating estrogen levels and mammographic density in postmenopausal women. Three studies found no association[386-388], one an inverse association[389] and four a positive association between

circulating estrogen (one or more forms) and mammographic density[390-393]. Three studies examined the association between plasma levels of estrogen and mammographic density in premenopausal women. None of these studies found an association between mammographic density and each of the estrogens measured including the study by Meyer et al. (1986)[394] which examined urine levels of estradiol, estrone and estriol.

Cumulative versus current endogenous hormone exposures and mammographic density.

The assumption underpinning these investigations is that estrogen levels at any given time are associated with mammographic density, yet evidence suggests that it is cumulative lifetime exposure to estrogen that is associated with mammographic density[395]. Tamimi et al. (2005)[386] reported the possibility that postmenopausal percent mammographic density might be more reflective of premenopausal circulating levels than postmenopausal levels.

A question to be asked is whether circulating estrogen levels are associated with change in mammographic density at any given period of time. A recent longitudinal study attempted to answer this question. Crandall et al. (2008)[396], examined the association between change in mammographic density and circulating estrone sulfate with HRT use. This US study followed up 428 women who had participated in the PEPI randomised controlled trial and had taken HRT. After adjusting for age, BMI, parity and a number of other risk factors, the investigators observed a 1.3% increase in mammographic density for every 1 ng/ml increase in estrone sulfate ($p<0.0001$) in women who had taken HRT. While Ursin et al. (2004)[339], using the PEPI cohort, observed a 2.95% increase in percent mammographic density for every 0.1 ng/ml increase in serum estrone level ($p=0.0003$) in women who used E and P hormone therapy but not estrogen only HRT. Crandall et al.[396], in the study reported above, observed a greater association between change in estrone sulfate level and change in mammographic density in women who used combined HRT compared to women who used estrogen only HRT ($p=0.05$).

Local tissue estrogen concentrations

Another suggestion for the lack of a clear association between mammographic density and circulating estrogens is the assumption that circulating estrogens reflect local tissue specific

concentrations of estrogens. According to Simpson (2003)[397], the levels of circulating estrogens and their precursors are unlikely to reflect the concentration of estrogen or the availability of its precursors in the breast tissue. It is possible that the concentration of estrogen within the breast provides a better reflection of the exposure to breast tissue, and it is the tissue specific estrogen concentrations that are likely to be associated with mammographic density. In the mouse model, paracrine (or local produced IGF-I), rather than endocrine concentrations have a more important role in mammary gland morphogenesis[398].

Endogenous estrogen and breast cancer risk

While there is inconsistency among the studies that examined the association between plasma estrogen levels and mammographic density, there appears to be greater consensus among studies that had examined the association between estrogen levels and breast cancer risk. A summary of these studies can be found in Chapter 3, Section 3.3.1. It is possible that circulating estrogens and mammographic density are independent risk factors of breast cancer. The study by Tamimi et al. (2007)[399] examined this proposal, and found circulating estrogens and mammographic density to be independently associated with breast cancer risk in postmenopausal women.

6.4.3.2 IGF-I and mammographic density

A number of epidemiological studies have examined the association between IGF-I and mammographic density. The findings of these studies are relevant to this PhD study because girls treated with high-dose estrogens for tall stature have been shown to have reduced circulating IGF-I levels throughout the duration of treatment. For a more detailed review of the nine cross-sectional and one prospective study of the association between IGF-I and mammographic density see Appendix 7.

Overall the evidence for an association between mammographic density and IGF-I is still inconclusive, but based on the larger of the studies summarised in **Table 6.6,** it appears that a positive association is likely, particularly in premenopausal women. These findings on mammographic density and IGF-I, at least for premenopausal women, are consistent with the effect of IGF-I on the proliferative activity of mammary tissue shown in animal[20, 400-402] and

primate[403] studies. The findings are also consistent with the positive association between IGF-I and breast cancer risk described earlier in Chapter 3, Section 3.3.4.3. It is possible that levels prior to menopause, when levels are typically higher, particularly during adolescence (see **Figure 6.7**), contribute more to mammographic density than postmenopausal levels. A prospective study by Verheus et al. (2007)[404] suggests that postmenopausal mammographic density is dependent on premenopausal levels of IGF-I. Based on the evidence above, it is plausible that treatment with high-dose estrogens for the treatment of tall stature in adolescent girls could reduce mammographic density. Treated girls have been shown to have reduced levels of IGF-I as described in Chapter 2.

6.4.3.3 Prolactin and mammographic density

Prolactin, similarly, has been shown to be positively influenced by mammographic density in postmenopausal women[197, 392, 405] and in one study, premenopausal women[394]. Though, some studies have not observed an association[390, 393] in postmenopausal women. The possible association between prolactin and mammographic density is relevant to this study because girls treated with high-dose estrogen have been shown to have higher than normal prolactin levels during treatment (See Chapter 2, Section 2.4.2.4). It is plausible then, that the increased circulating prolactin observed in girls treated with high-dose estrogens for tall stature might lead to increased levels of mammographic density.

6.5 Adolescent exposures and mammographic density

Evidence presented above suggests that exogenous hormone treatments are associated with mammographic density in the adult. The hormone induced changes in mammographic density also appear to be associated with change in circulating estrogens. However, this change in mammographic density appears to be short-lived; only lasting the duration of treatment. Whether estrogen treatment in adolescence affects mammographic density and whether this effect, if any, lasts to adulthood, is unknown. The research literature was reviewed for any evidence of adolescent exposures affecting mammographic density through to adulthood. A summary of the studies that were identified is presented below.

6.5.1 Age at menarche and mammographic density

Age at menarche has been associated with mammographic density in some studies[302, 406-409], but not others[360, 361, 410-414]. In all but one of the studies that observed an association, age at menarche was positively associated with mammographic density or high-risk parenchymal patterns. Gram et al. (1985)[415], in their study involving 3,640 Norwegian women, observed a dual response depending on menopausal status. They observed an inverse association in postmenopausal women, and a positive association in premenopausal women. Either way, it appears that hormonal exposures in adolescence might affect mammographic density later in life.

6.5.2 Adolescent lifestyle factors and mammographic density

A number of studies examined the association between adolescent lifestyle factors and mammographic density including alcohol, smoking, diet and physical activity. Vachon et al. (2005)[416] found no influence of alcohol intake during adolescence on mammographic density in adulthood, while studies have observed positive associations with current alcohol use[305, 417, 418], but not lifetime use[417], as an adult.

Sellers et al. (2007)[419] found no association between physical activity levels in adolescence with mammographic density in adulthood. However, some studies[420-423] have observed an association between adult levels of physical activity with percent density while others have not[305, 424-428]. Jeffreys et al. (2004)[360] observed an inverse relationship between age at start of smoking and percent mammographic density in a cross-sectional study involving 628 women attending the University of Glasgow Health Service. They observed a positive association between age when smoking started and mammographic density. The odds of having a high-risk density category (>25% density vs <25% density) for each year of age that smoking was started was 1.08 (95% CI: 0.99 to 1.16, P for trend 0.058) after adjusting for HRT use, OC ever use and adult BMI. These findings are consistent with the association observed between smoking exposure as a child and breast cancer risk in postmenopausal (but not premenopausal) women[429]. In this case-control study by Ahern et al. (2009)[429], postmenopausal (but not premenopausal) women exposed to cigarette smoke as a

child had a modestly increased risk of breast cancer (adjusted OR 1.8: 95% CI: 1.0 to 3.3) compared to women who were never passively exposed at any age.

Of three reported studies that have investigated the association between adolescent diet and mammographic density, two[419, 430] found no association while Maskarinec et al. (2006)[329] found early life soy intake (self reported as an adult) to be associated with lower mean percent density (–8.6%, p=0.07) in Japanese women (mean age 57 years of age), suggesting that adolescent exposures can have lasting effects on mammographic density.

6.5.3 Childhood anthropometric factors and mammographic density

A number of childhood anthropometric factors have also been shown to be associated with mammographic density as an adult. These include birthweight[431], childhood height[419], weight[419, 426] and height velocity[406]. The two studies[431, 432] (of six) that examined the association between percent density and birthweight using a continuous measure of percent density found a positive association in postmenopausal women. No association has been observed in premenopausal women[431]. The two reported studies[432, 433] that examined the association between birth-length and mammographic density found no association. Childhood height[406, 419] at particular ages, and growth velocity[406] have been positively associated with mammographic density. Interestingly, McCormack et al. (2003)[406] found significant but opposing associations with higher Wolfe grade density for height at ages 2 and 11; adjusted OR 1.13 (95% CI: 1.01 to 1.26) (p=0.03) (n=1033), and OR 0.89 (95% CI: 0.80 to 1.00) (p=0.04) (n=1090), respectively. No significant association was observed for ages 4, 7, or 15 years.

McCormack et al. (2003)[406], Sellers et al. (2007)[419] and Samini et al. (2008)[426] observed an inverse association between childhood BMI or weight and mammographic density. The one study that examined the association between age at maximum height and mammographic density found no such association, though it relied on self reported age at maximum height as an adult. A more detailed summary of these and other reported studies that have examined the association between childhood anthropometric factors and mammographic density is in Appendix 8.

In those studies observing an association with mammographic density as an adult, it would be expected that an association would also be present in adolescence. Few studies have directly measured mammographic density in adolescent girls which is not unexpected given the difficulty of exposing adolescents to mammogram related radiation. Magnetic resonance imaging (MRI)[282] and dual-energy x-ray absorptiometry[434] are two methods that have been used for the measurement of mammographic density in young women and are promising techniques for future studies.

Of particular interest is the study by Boyd et al. (2009)[282]. They examined the association between serum hormone levels and percent water content of the breast in 260 women aged 15-30 years (median age 18 years) using MRI. The water content of the breast corresponds with the fibro-glandular tissue, which is mammographically dense. In this study, percent water content measured using MRI was highly correlated with percent mammographic density in the mothers of the young women (Spearman correlation coefficient = 0.85)[282]. They found growth hormone and sex hormone binding globulin; but not IGF-I, estradiol or progesterone, to be associated with mammographic density. Whether or not hormone levels measured at a young age remain associated with mammographic density later in life is unknown.

6.6 Overview

This chapter began by describing mammographic density and the different methods of density measurement, and then followed with a review of studies that have examined associations between mammographic density and breast cancer risk. Many studies have examined this association and have subsequently been reviewed separately by Boyd and colleagues (1998)[4] and McCormack and dos Santos Silva (2006) [288]. In their meta-analysis of quantitative studies, Boyd et al.(1998)[4] showed that women with dense tissue in more than 60–75% of the breast had 4–6 times greater risk of breast cancer than those with a lower proportion of dense tissue after adjustment for relevant covariates[4]. While this risk gradient was derived from multiple-studies, there were disparities between studies. McCormack and dos Santos Silva[288] explored possible reasons for these disparities and observed a difference between incident and prevalent breast cancer studies. Between these two types of studies,

they found breast cancer risk to be 4–5 fold larger in women with >75% dense breasts compared with women with less than 5% dense breasts[288].

The majority of research studies on mammographic density have used the relative measure of density (% density). The studies that also used the absolute measure of dense area have also observed a positive association with breast cancer risk[289-293]. In a comparative assessment, Torres-Maija and co-investigators found absolute density to be a stronger predictor of breast cancer risk than percent mammographic density[290]. This has been supported by others[289, 294] but not all[292, 295, 296]. Mostly, it appears that the two measures are similar in their association with breast cancer risk[292, 295, 296].

This chapter also described the different methods that have been used to measure mammographic density. Qualitative methods of mammographic measurement include the Wolfe grading system, and the BI-RADS scaling system. These two qualitative systems use four categories of density classification, and consequently, are only able to detect large changes or differences in density [272]. They are measured by visual estimation and are more subjective than the quantitative measures of density that include visual estimation, computer assisted thresholding and planimetry. There is no gold standard in mammographic density measurement. As described in detail in Section 6.2.2.4 above, the comparative inter-rater and intra-rater reliability assessments of qualitative and quantitative methods suggest that continuous quantitative measurement of mammographic density (as percent or absolute density) produces the highest reliability scores. The computer assisted technique is less time consuming than planimetry (both in training and execution), and in the case of visual estimation, is less subjective. It is the method used and described in the following chapter.

This chapter also explored the evidence of hormonal influences of mammographic density (both exogenous and endogenous). A review of cross-sectional and longitudinal studies suggest that exogenous hormone exposures in adulthood (e.g. HRT) and endogenous hormone levels (e.g. IGF-I) have been associated with mammographic density. While the cross-sectional studies cannot measure change in density directly, they can measure differences in mammographic density between groups (e.g. between users and non-users of HRT), or to measure associations (e.g. between mammographic density and duration of HRT

use). The cross-sectional studies consistently reported increased levels of mammographic density in HRT users compared to non-users. The longitudinal studies generally observed an increase in HRT compared to baseline or for one study, a reduction in the rate of age-related decline in mammographic density. Not all studies supported these findings however. The randomized placebo controlled studies also differed in their findings. Direct comparisons between the studies are difficult due to the differences in length of follow-up, levels of adjustment, and type of hormones used.

One consistency that does exist across studies is the larger effect on mammographic density observed with combined estrogen and progestagen compared with estrogen only HRT formulations. The longitudinal studies using quantitative continuous measures have observed mean increases in mammographic density between 2.8% and 7.7% after estrogen and progesterone combined HRT exposure, compared with 1.2–1.3% after estrogen only HRT exposure.

HRT appears to only increase mammographic density in some women. In the majority of the longitudinal studies using qualitative or categorical measures of mammographic density, the proportion of women who experienced an increase in mammographic density following exposure to combined HRT ranged from 2 to 68%, with the majority of studies reporting between 20% and 40%. These figures are much larger than the proportion who experienced an increase in mammographic density following estrogen only HRT (3.9 to 22%). It is unclear why HRT influences mammographic in some women and not others. The qualitative or categorical measures of density might not be sensitive enough to identify increases within the density categories. Many of the studies using categorical or qualitative measures of mammographic density could not detect changes less than 10%. Laya's finding of 73% would reduce to 32% if changes less than 10% (41% of reported changes) were not included.

Overall the evidence for an association between mammographic density and IGF-I is still inconclusive, but based on the larger of the studies, it appears that a positive association is likely, particularly in premenopausal women. These findings on mammographic density and IGF-I, at least for premenopausal women, are consistent with the effect of IGF-I on the

proliferative activity of mammary tissue shown in animal[20, 400-402] and primate[403] studies. While caution is required when translating findings in the animal model to humans, they can support similar findings in human studies. The findings are also consistent with the positive association between IGF-I and breast cancer risk described earlier in Chapter 3, Section 3.3.4.3. It is possible that levels prior to menopause, when levels are typically higher, particularly during adolescence contribute more to mammographic density than postmenopausal levels. A prospective study by Verheus et al. (2007)[404] suggests that postmenopausal mammographic density is dependent on premenopausal levels of IGF-I.

Based on the evidence presented in this chapter, it is plausible that treatment with high-dose estrogens for the treatment of tall stature in adolescent girls could reduce mammographic density. Longitudinal studies have been described that appear to show treatment with high-dose estrogens reduce levels of IGF-I as described in Chapter 2. However, it is important to note that these studies are not placebo controlled.

A number of adolescent factors were found to be associated with mammographic density as an adult (e.g. age at menarche, anthropometric variables). While these studies suggest that hormone exposures influence mammographic density, no study has examined the effect of exposure to high-dose estrogens in adolescence on mammographic density as an adult. The following chapter outlines the research study that explores this association.

Box 6.1: Key points from the literature in Chapter 6.

KEY POINTS FROM THE LITERATURE: CHAPTER 6

- Mammographic density is a strong risk factor for breast cancer.
- There are a number of methods for measuring mammographic density, with the computer assisted thresholding method the most reliable method for quantitative continuous measures of density.
- Cross-sectional and longitudinal studies suggest that exogenous hormone exposures in adulthood (e.g. HRT) and endogenous hormone levels (e.g. IGF-I) have been associated with mammographic density.
- HRT appears to only increase mammographic density in some women.
- Increases in mammographic density with HRT use coincides with breast pain in some women, which is also associated with increasing breast cancer risk.
- Studies suggest that HRT reduces the age-related reduction in mammographic density and that the difference in density between HRT and non-HRT users increases with increasing age.
- HRT has been shown to increase mammographic density with the combined estrogen and progestagen formulations having a stronger effect than estrogen only formulations. Combined formulations containing a continuous progestagen have been shown to have a stronger effect than those that use a cyclic progestagen.
- A number of adolescent factors have been associated with mammographic density as an adult (e.g. age at menarche, anthropometric variables).
- While these studies suggest that hormone exposures influence mammographic density, no study has examined the effect of exposure to high-dose estrogens in adolescence on mammographic density as an adult.

7: THE LONG-TERM EFFECT OF HIGH-DOSE ESTROGEN EXPOSURE IN ADOLESCENT GIRLS ON MAMMOGRAPHIC DENSITY

7.0 Introduction

In Chapter 6, it was shown that mammographic density is a well established risk factor of breast cancer and is influenced by exogenous and endogenous hormone levels in adulthood and adolescence. The chapter also presented evidence suggesting that adolescent exposure to high-dose estrogens may lead to changes in mammographic density in adulthood.

It is unclear what the direction would be for an association between high-dose estrogen exposure in adolescence and mammographic density. HRT use has been associated with an increase in mammographic density. Consequently, estrogen plus cyclic progestagen, the typical treatment regimen in girls treated for tall stature, might result in an increase in mammographic density in adulthood. An alternative scenario is a reduction in mammographic density. Treatment for tall stature with high-dose estrogens has been shown to attenuate IGF-I levels in girls. Some of the studies presented in Chapter 6 demonstrated a positive association between circulating IGF-I levels and mammographic density. Similarly, earlier age at menarche is associated with decreasing levels of mammographic density. Treatment induces menarche in girls who have not yet reached menarche at the start of treatment. Consequently, it is possible that mammographic density is lower in women treated with high-dose estrogens as an adolescent compared to women who were not treated but assessed for tall stature.

The Tall Girls Study cohort provided a unique opportunity to examine the influence of treatment with high-dose estrogens in adolescence on mammographic density in adulthood. NHMRC research funding was sought for a second follow-up of the Tall Girls cohort and a project grant was successfully attained. The following sections describe the methodology and results of this research.

7.1 Study Aim

This study aimed to examine the association between exposure to high-dose estrogen in adolescence and mammographic density as an adult using a second follow-up of the Australian Tall Girls cohort.

7.2 Methods

7.2.1 Eligibility

This retrospective cohort study included women who had previously participated in the Tall Girls Study[25], were 40 years of age or older, and had stated that they were willing to be re-contacted for further research. As described in Chapter 3 and elsewhere[25], women were eligible to participate in the Australian Tall Girls Study if, between 1953 and 1993, they had obtained a medical opinion about their tall stature during adolescence and had a radiological assessment of their skeletal age in order to predict their adult height; they included women who were treated with high-dose estrogens and women who were not treated.

7.2.2 Recruitment

7.2.2.1 Follow-up 1

In follow-up 1, most of the women were identified through the records of one paediatric endocrinologist. Other treating paediatricians (n=50) were identified through professional networks and by treated women themselves. The paediatricians were sent letters requesting their permission to identify eligible participants. Of those approached, only three were able to assist. As previously reported[25], the remainder did not assist because they no longer held records or could not readily identify eligible individuals (n=25), were unwilling to assist (n=2), were deceased or unwell (n=11) or could not be contacted (n=9).

A total of 1,432 eligible participants were identified: 1,248 from medical records (1,222 from one paediatric endocrinologist) and 184 from self-referrals. Self-referrals included women who were members of the advocacy group Tall Girls Inc. and women who contacted the study investigators directly as a result of publicity about the study. Of the eligible identified, 1,243 women were traced and invited to participate in the study. Of these, 371 treated (72% of those traced) and 409 (56%) untreated women provided their consent, and completed a postal questionnaire and telephone interview in 2002–2003.

7.2.2.2 Follow-up 2

This study of mammographic density involved a second follow-up of women in the Tall Girls Study cohort who were aged 40 years and over and therefore eligible to have a free mammogram as part of the national breast screening program. Of the 780 women (371 treated and 409 untreated) who had participated in the first follow-up, 517 (263 treated, 254 untreated) were eligible to participate in this second follow-up.

The contact details of women eligible for the second follow-up study were checked and updated with information from the electronic electoral roll, and on-line telephone directory. Of the 517 eligible, 483 were successfully traced and contacted. A consent form, letter of invitation, study information brochure and mammogram release form was sent to the women inviting them to participate in the study. The study background and aims were outlined and a letter of invitation asking women to be interviewed by telephone and provide the study with access to a mammogram they may have had in the previous two years, or to have a mammogram at their closest publicly funded breast cancer screening service (BreastScreen) and provide us with access to the film for scanning. The mammogram release form asked for information about the whereabouts of the last mammogram and when it was performed. To assist us in retrieving films and information from the BreastScreen services, women were also asked to provide their permission to allow BreastScreen to provide us with information about their BreastScreen attendances. This was later sent to the relevant service holding the film. See Appendix 9 for a copy of the consent form, study invitation letter, information brochure and mammogram release form.

If the women did not respond by mail or phone, a follow-up phone call was made within two weeks to check that they had received the study promotion material, and if they had, whether they were interested in participating in the study. If women declined participation, they were thanked and not contacted again. If women registered their interest and stated that they intended to return their consent forms to us, they were followed up again by telephone if we did not receive their consent forms within approximately a month.

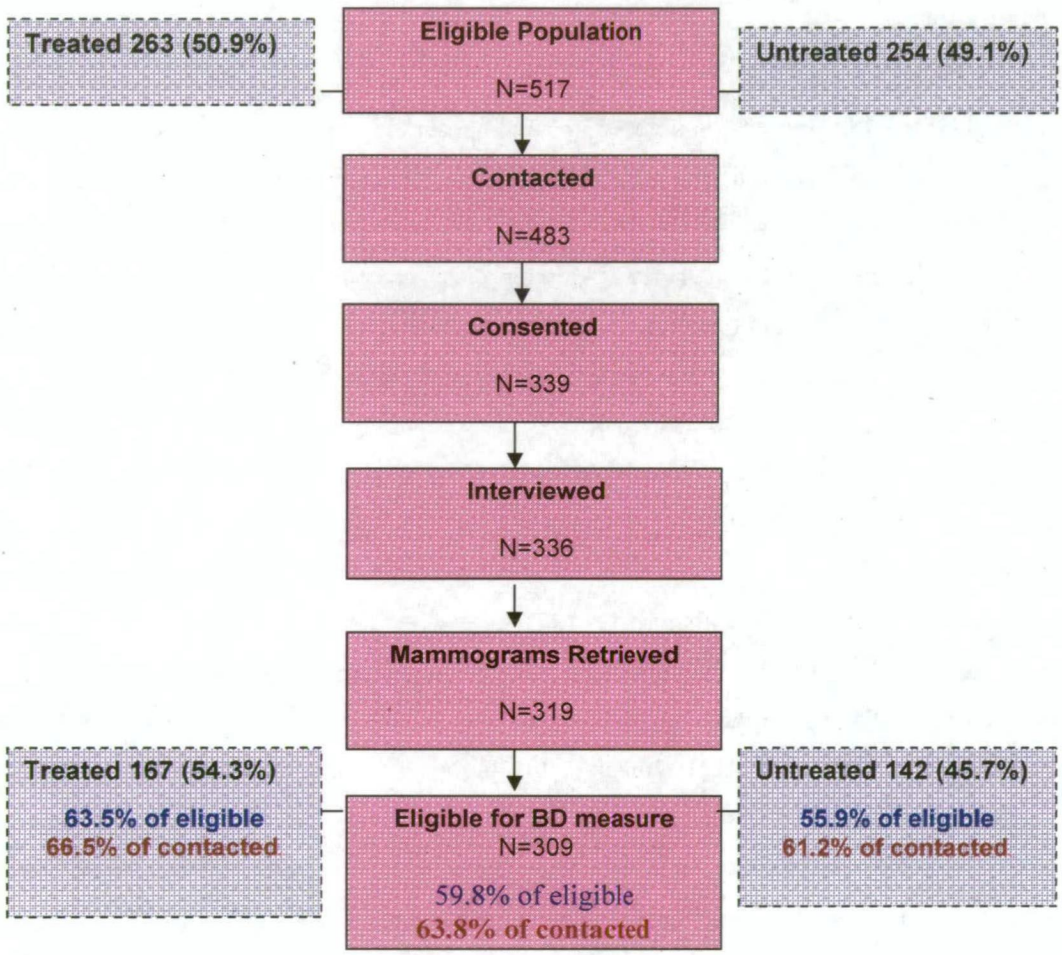
Up to three phone-calls were made to study participants to remind them to return the completed consent form if they wanted to participate in the study. Of the women who had

returned a consent form, up to two reminder phone calls were made to remind them to book a mammogram if the BreastScreen service had no record of them having presented for a mammogram.

Of the 483 women contacted, 339 (70%) provided their written consent to participate (185 treated, 154 untreated) and 336 were subsequently interviewed by telephone in 2006–2007. The three women who consented but were not interviewed were unable to be contacted by phone to organise an interview. A letter was sent to these women to organise the interview, but they did not respond.

The mammograms of 319 participants were obtained from BreastScreen (77% of treated, 73% of untreated), from private screening services directly or from participants themselves, if they held them. Seventeen women who were interviewed did not have a mammogram. Women's reasons for not having a mammogram at a BreastScreen service included, lack of time, inflexible access to BreastScreen services in some rural areas and in one case, a recommendation from her GP not to have a mammogram because of the exposure to radiation. Of the 319 mammograms obtained for the study, 309 were eligible for breast density measurements (167 treated, 142 untreated). Mammograms were ineligible for density measurements if the woman had breast surgery in both breasts before the mammogram (treated=4, untreated=3), if tamoxifen was used within two years prior to the mammogram (treated=1, untreated=1), or if the image was of poor quality (treated=1). **Figure 7.1** summarises the numbers of women identified, traced, interviewed and included in the final mammographic density study.

Figure 7.1: Flow chart of recruitment of study participants



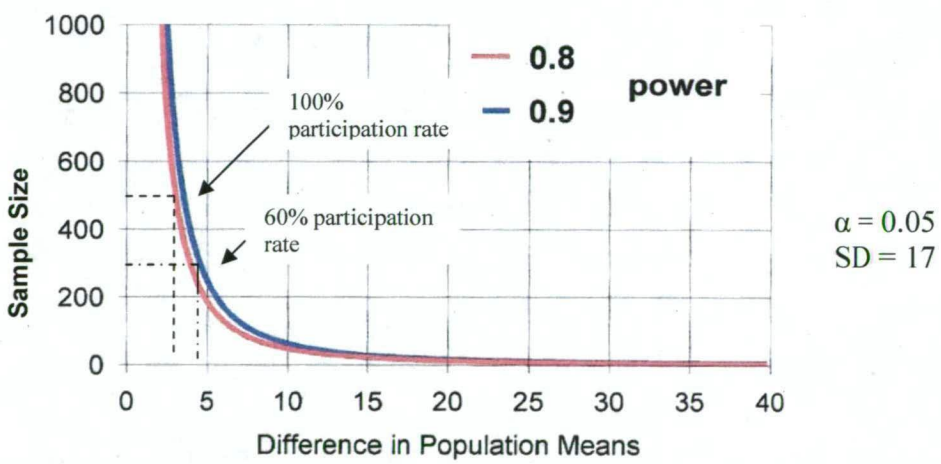
7.2.3 Sample size determination

As the population of eligible women was known from the outset, power calculations were undertaken to identify the difference in percent density that would produce statistical significance if 60% of the eligible population participated in the study. To do this, the standard deviation for percent density for the eligible population was needed. A search of the literature identified the variance derived standard deviation (SD) of percent mammographic density across the age range 40–70 (mean PMD is 40% at age 40 and 30% at age 70), and adjusted for the covariates age, BMI, age at menarche, parity, number of live births, age at

first birth and cessation of periods, to be 17[395]. This standard deviation is consistent with other reported population standard deviations for a similar age range[435, 436]. The age range of participants in the study is narrower than those above (40–59 years), so the SD estimate will be conservative.

Sample sizes from participation rates ranging from 60–100% with alpha at 0.05 and power of 0.8–0.9 would allow the study to detect a 3–4% difference in mean percent mammographic density between the treated and untreated groups (**Figure 7.2**). Given that all of the women in the sample population participated in the previous follow-up and had stated their interest in being involved in further research, it was accurately anticipated that at least 60% of eligible participants would be recruited for this new investigation.

Figure 7.2: Sample size and difference in population means for study powers of 0.8 and 0.9.



7.2.4 Measurement of outcomes: mammographic density

7.2.4.1 Interactive computer thresholding method

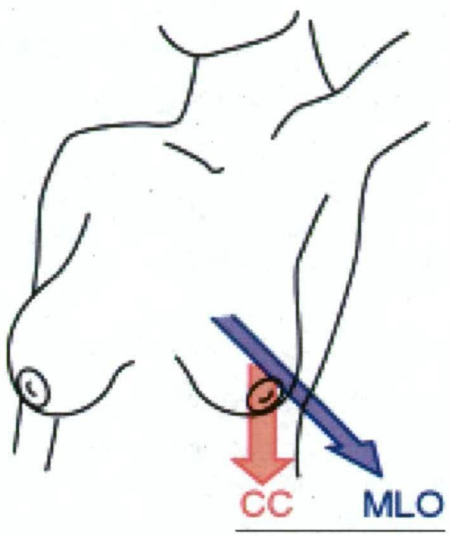
A number of methods are available to measure mammographic density. These were summarised earlier in Chapter 6 (see Section 6.2). The method used in this study was the interactive computer thresholding method using Cumulus software developed at the University of Toronto and described elswhere[437]. This method provided a continuous quantitative measure of percent density, dense area, total area and non-dense area, which is preferable to the qualitative forms of measurement, and less subjective than the visual estimation of density. The equipment and opportunity for training was provided at the Centre for Molecular, Environmental, Genetic and Analytic Epidemiology (MEGA) at the University of Melbourne. Training was provided by radiologist, Jenny Cawson, and PhD student, Jennifer Stone, both of whom had extensive experience in the use of the Cumulus software and were trained by Professor Norman Boyd in Toronto.

7.2.4.2 Cranio-caudal view

The left and right cranio-caudal mammograms of each study participant were collected and digitised using a Lumisys 85 scanner housed at the University of Melbourne. The cranio-caudal view of the breast is taken from above a horizontally compressed breast. The other common view is the medio-oblique (MLO) view which is taken from the side of a diagonally-compressed breast. See **Figure 7.3** of an illustration of the cranio-caudal perspective of mammogram compared to the medio-lateral perspective.

Two state BreastScreen services did not allow the interstate movement of mammograms. To accommodate this, the scanning equipment was transported to these states where the films were scanned by Jennifer Stone or myself.

Figure 7.3: Cranio-caudal and medio-lateral mammogram views.
Sourced from *http://breastcancer.about.com/od/mammograms/a/mamm_views.htm*



The right breast was preferentially used for mammographic density measurements. If the right mammogram was unavailable or was not suitable for density measurement (see eligibility criteria, Section 7.2.2.2 above), the left was used.

According to Byng et al. (1996)[438], representative information on mammographic density is satisfactorily provided in a single view. Pearson correlation coefficients of between 0.86–0.96 have been observed between the right and left breast and between cranial-caudal and medial-lateral oblique views for quantitative measurements of mammographic density. Yaffe and colleagues[439] also examined the consistency in mammographic density results between four views of the breast (left and right cranio-caudal and medio-lateral oblique) and concluded that studies of mammographic density can use any one of the four views.

7.2.4.3 Masking of image

Each film had to be masked to hide patient identifying information and to remove any white parts of the film outside of the breast area that would be picked up as 'dense' material. Masked areas appeared red on the film. The masking process also involved outlining the back of the breast. Pectoral muscle appears white on the screen and was excluded from analysis.

7.2.4.4 Measuring density

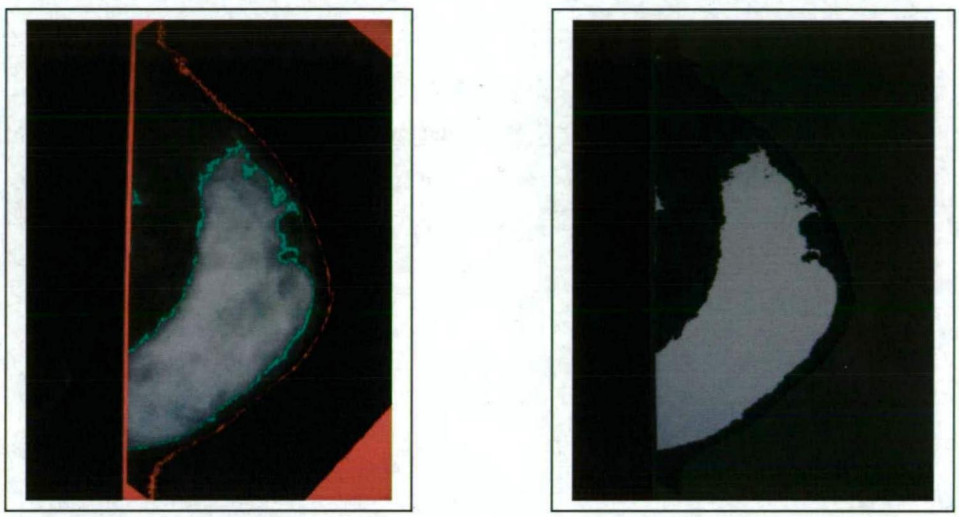
Once all the films were masked, breast density measurements were undertaken in eight batches of 50–100 left and right cranio-caudal films by one reader (myself). The films were randomly placed in batches. Masking and randomisation ensured the reader was blinded to the treatment status of the women.

The breast was outlined using a sliding thresholding scale on the screen with the aide of a mouse. This thresholding scale simultaneously adjusted the contrast of the breast with the dark background, detecting the difference between the two. Once the breast was outlined, a second thresholding scale was used to clearly delineate the dense area of the breast from the non-dense area, and a third sliding scale used to outline the dense area pixels. After the thresholds were set, the verification button was pressed to observe the areas of the breast that would be register as dense. This was performed for each film. This allowed the 'masker' to see if all sections of the border and pectoralis were hidden. If they were not, the masking was adjusted accordingly.

The first image in **Figure 7.4** highlights the total breast area and dense area boundaries. The red and green lines define total breast area and dense area respectively. Red areas outside of the breast area are there to mask identifying information and/or white edges of the film that may otherwise be picked up in the analysis. The second image in **Figure 7.4** shows the verified image. In this image, the white area that would be included as dense breast tissue is highlighted.

Figure 7.4: Masked (A) and verified (B) images of mammograms.

A. B.



Masking and the measurement of breast density of the films occurred over a two week period.

Mean of two sets of reads

The films were all re-read twice on separate occasions. It was felt that some 'settling in' occurred with the first read of the batches, and consequently the last two reads of the batches were used in the analysis.

Spearman's Rho of 0.95 was calculated for all data in Reads 2 and 3. Intra-observer within subject reliability was measured by using the intra-class correlation (ICC) coefficient using the loneway command in Stata. The ICC was 0.93 for both percent density and dense area and the means of these two sets of reads were used in the analysis.

In addition, the opportunity arose for a repeat analysis to be undertaken using the mean of two sets of reads performed by a second experienced reader (Jennifer Stone) 12 months later. These data are reported separately in the results section.

Calibration of the scanner

Films were scanned over an 11 month period. While there would be no systematic bias in the order of the reads it was important that the optical density and pixel grey values were constant across the reads. These parameters were measured after each scanning batch and compared with the previous batch. No significant variation occurred between the batches. See Appendix 10 for the plot of optical density and pixel grey values over time.

Films used in the analysis

The right mammogram was used for 93.0% treated and 92.8% untreated women, unless surgery, cancer or benign breast disease had occurred in that breast, in which case the left film was used. The use of the contralateral film in such circumstances has been performed elsewhere[437]. See Appendix 11 for a plot of percent mammographic density of the right breast with benign breast disease against the corresponding left non-diseased breast of the same woman. A Spearman coefficient of 0.99 was calculated between the two sides.

If the woman had a history of benign breast disease in both breasts, and no surgery, the right breast was used (n=10 treated, n=3 untreated). One treated participant (0.6%) and three untreated participants (2.1%) had a history of breast cancer in the breast not used to measure density.

Transformation of mammographic density data

The outcome variables dense area, non-dense area, total breast area and percent density were treated as continuous variables. A breast area of 1 cm^2 is equal to 6.67 x 10^{-4} pixels. Pixels were converted to cm^2 by multiplying the number of pixels by 0.000676 for dense area, non-dense area and total breast area.

The distribution of each of the dependent variables was checked and if the data were skewed, a Box-Cox method[257] was used to identify the most appropriate transformation approach to ensure the data fulfilled the criterion of normality for linear regression analysis.

7.2.5 Measurement of exposures: treatment

Detailed information on treatment characteristics of these women who participated in the second follow-up are described in the results section below.

Treatment information was extracted from the medical records of women who provided consent and for whom records were available (67% of treated and 92% of untreated women) or was self-reported by women in the postal questionnaire used in the first follow-up. The majority of women were treated or assessed by one paediatric endocrinologist (81%). More records were available for untreated women because a greater proportion of them (97%) compared with treated women (68%) were sourced from one endocrinologist who retained and allowed access to the medical records. More treated women self-referred to the study and had been treated by other endocrinologists whose records could not be accessed.

Type of treatment, and start and end date of treatment (from which duration of treatment was calculated) was collected from the records. If records were not available, women were asked in the postal questionnaire the name of the drug that they took (1=DES, 2=EE, 3=Other, 4=Not sure/can't remember name) and how old they were when they started treated and completed treatment (years and months).

Age at menarche and Tanner Stage of the Breast was also collected from the medical records. Start of treatment in relation to age at menarche and Tanner Stage were derived from this data.

7.2.6 Covariates

Potential confounders were identified from the research literature, through being either associated with mammographic measure(s) and/or breast cancer risk, and being measured using the questionnaires and other data sources (See Appendix 12 for a list of covariates collected and analysed in this study).

While there are a few inconsistencies across the studies, it is apparent that age[305, 306, 325, 328, 359, 407, 409, 413, 440-443], BMI[305, 325, 328, 359, 360, 407, 409, 413, 442, 444] and parity[305, 306, 328, 359, 360, 407, 409, 413, 440-444] are inversely associated with percent density. Other potential confounders include age at menarche[302, 328, 359, 407, 409, 444] and age at first livebirth[328, 360, 409, 445, 446] and history of breast biopsy[306, 444-447] which have been reported to be positively associated with mammographic density, and menopause[325, 328, 409, 444, 448] which has been reported to be negatively associated. Associations have also been reported between mammographic density and smoking[305, 360, 449], alcohol[417, 444, 450], diet[444, 451-453], height[446], breastfeeding[444], and having had a first degree relative with breast cancer[454]. The association between exogenous hormone use and mammographic density has been described in detail in Chapter 6 (Section 6.4.1).

At the time the questionnaire was being developed, some factors did not appear to be associated with mammographic density but had been shown to be associated with breast cancer risk (e.g. fertility drug[455] and aspirin use[456]). Since mammographic density is also associated with breast cancer risk, these data have been collected as they may influence any association between treatment and mammographic density. Similarly, while no studies appear to have investigated the link between hormone treatments for endometriosis or menstrual disorders and mammographic density, data was collected on these factors. It is possible that any difference in mammographic density between women treated with high-dose estrogens in adolescence and untreated women might be due to other hormonal exposures. For instance, data from the Tall Girls Study (not published) suggests that treated

women are at higher risk of endometriosis compared to untreated women. It is possible that women who have had a history of endometriosis have taken hormonal medication. Information on hormonal medications other than for contraceptive, HRT, or fertility use were therefore collected.

7.2.6.1 Covariate data collection tools

Data on covariates were collected from a questionnaire used in follow-up 1 and follow-up 2. The follow-up 2 questionnaire was developed to update information collected in the first follow-up and to collect additional information. A copy of the questionnaires can be found in Appendices 3 (postal) and 4 (CATI) for follow-up 1 and Appendix 13 (CATI), for the second follow-up.

Questionnaire development

The follow-up 2 questionnaire was derived from the Australian Twins and Sisters Breast Density Study[457] questionnaire with some amendments. Questions only relevant to twins were removed, while additional questions about hormone use for reproductive problems were added. Some questions that were used in the follow-up 1 study questionnaire were repeated in this questionnaire to update previously collected data (e.g. breastfeeding and pregnancy data).

The follow-up 2 questionnaire was developed for computer assisted telephone interview (CATI). This process required extensive testing and piloting. An Access 2000 database was used as the platform for the CATI questionnaire. The questionnaire was piloted with women not associated with the study. Questions were fine-tuned, and it was again piloted. Two interviewers (myself and Shirley Catchpole) performed the telephone interviews.

Questionnaire questions

Data on the following covariates were self-reported by participants at follow-up 1 only: age at last menstrual period (CATI) and highest education level achieved, and marital status (postal questionnaire).

Data on the following covariates were self-reported by participants at follow-up 1 (by postal questionnaire or CATI) and updated at follow-up 2 (by CATI): reproductive history (including pregnancy outcomes, breastfeeding history and menstruation); history of breast and gynecological disorders (e.g. has a doctor ever told you that you had/ have you ever been diagnosed with endometriosis, uterine fibroids, ovarian cysts, benign breast disease and breast cancer); use of hormonal medications, hormonal contraceptives, hormone replacement therapy and hormones for infertility; smoking and alcohol consumption, and current height and weight from which BMI was derived (kg/m^2). At follow-up 2, family history of breast cancer, and country of birth was also collected. Smoking and alcohol history questions were derived from the Australian Longitudinal Study on Women's Health (Women's Health Australia).

The CATI questions on reproductive and gynecological health in follow-up 1 and later updated in follow-up 2 were derived from large-scale studies of reproductive health and health outcomes in women exposed to DES conducted in the USA by Wilcox[458] and Baird[459]. At follow-up 1 information on reproductive history was collected by asking women for each pregnancy: in what month and year did your pregnancy end, how did the pregnancy end, how many weeks did this pregnancy last, did you commence breastfeeding this baby, how long did your baby have breast milk only, and how long did you breastfeed this baby all together. Follow-up 2 updated this information by asking, since the last interview (date of interview at follow-up 1 was provided to participant), have they been pregnant, and for each pregnancy what month and year the pregnancy ended, how many weeks pregnant were they when the pregnancy ended, how the pregnancy ended, and how long they breastfed if they did.

Women were asked if they had ever taken prescription estrogens, progesterone or other female hormones for menopause, that is, prescription hormone replacement therapy or HRT not including birth control pills or hormonal contraceptives, whether they were still having periods when they first took HRT and how long after their last period they took HRT. To obtain information on overall HRT exposure, women were asked when they stopped and started HRT use and whether they commenced again after that. The questions continued this way until they answered that they did not commence HRT again after stopping or they were currently using HRT. A similar format of questioning was used to measure cumulative oral contraceptive use.

The questions on HRT use in follow-up 2 asked women for the names of the hormonal replacement therapies if they could recall these and whether they knew whether they contained an estrogen and/or progestagen. The hormones were later categorised into estrogen only or estrogen and progestin combined HRT formulations. This information was derived from a list developed for a recent Australian study by Nickson and Kavanagh[460], on the reliability of recall of menopause therapy. This list was developed by cross-referencing prescription names listed on the Australian Pharmaceutical Benefits Scheme, and the internet for non-prescription medicines.

At follow-up 2, menstrual history was collected by asking women what age they were when they had their last period and why their menstrual periods stopped (from which menopausal status was derived). Women were defined as postmenopausal if the time since their last period was ≥52 weeks and a) they had not started HRT before their periods ended or b) they started HRT before their periods ended and they were ≥55 years of age. Recognising that some women would have not had a period for ≥52 weeks because of hysterectomy (while retaining one or both ovaries), endometrial ablation, IUD, or hormone implants, we also-examined menopausal status using a definition that considered these women to be premenopausal unless they were ≥55 years of age.

Additional childhood anthropometric measures were collected and analysed as described in Chapter 8 (see Appendix 12 for a list of these measures). The aim, methods and findings of these data analyses are reported in Chapter 8.

7.2.7 Ethics approval

7.2.7.1 Ethics approval granting bodies

The study was granted institutional research ethics committee approval from the Southern Tasmania Health and Medical Human Research Ethics Committee (H0008334), ACT Health HREC (ETH.5/06.313), Department of Health (SA) HREC (14/12/05), Cancer Institute NSW (2006/06/003), BreastScreen Victoria Research and Evaluation Committee (25/10/05), BreastScreen Queensland Monitoring Research and Evaluation Sub-committee (0243–0271-003), and the Uniting Care Health HREC Queensland (20/6/07). Copies of these approvals are provided in Appendix 14. Prior to obtaining formal ethics approval from each of the state BreastScreen services and relevant bodies, discussions were made with the primary BreastScreen service within each state to discuss the protocol for the retrieval and return of mammograms.

7.2.7.2 Ethical issues

A number of ethical issues needed to be taken into consideration in the study. These are described below.

Mammographic screening

The mammographic screening process had the potential to cause discomfort to women. Also, having a mammogram involves exposure to ionising radiation. This exposure was considered to be minimal. BreastScreen mammogram is routinely available to Australian women over the age of 40 years and radiation safety issues are managed by the BreastScreen program.

Data handling and management

The digitised images are stored with the personal information provided on the mammographic films as the scanner will not function with anything attached to the film which could be used to conceal the personal details. BreastScreen Service mammograms are

routinely identified. To ensure confidentiality of the digitised images was maintained, the digitised images were only viewed by the investigators performing the density calculations.

A de-identified data file, with a coded ID number was used for the analysis. If necessary, it is possible to link the ID code to the original identifiers and identify the individual to whom the information relates. While it is possible for the data to be re-identified, it is unlikely that this will be necessary.

The file containing names and ID numbers was kept separately, and password protected. The back up and archived copies density calculations, and interview data was saved on a password protected database and stored at Menzies Research Institute. On completion of the study the digitised images were archived on a separate password protected database at Menzies Research Institute.

Reporting the findings

The identity of participants was known to the investigators but not to others outside the research team. Participants are not identifiable in presentations or publications. Names have been stored separately from data on completion of the data collection.

Informed consent

Consent was required to participate in the study. This required two consent forms – giving consent to participate in the study (being interviewed and allowing the study to access a mammogram they had in the previous two years, or if they did not have one, to have a mammogram at a local BreastScreen service). A second form, indicated consent to allow the investigators to access the women's mammograms. A copy of this signed form was sent to services holding the mammogram. Consent was also required of women for the study to access medical records. This consent was obtained in follow-up 1. Consent forms are contained in Appendix 15.

An information brochure was provided with the consent forms to ensure women had a clear understanding of the aims of the study and the procedures involved. The information brochure is in Appendix 9.

7.2.8 Data analysis

A range of descriptive and inferential data analyses were performed as described below. Stata software (version 9) was used for all analyses.

7.2.8.1 Descriptive analysis

Descriptive analysis of the data included scatter plots and box and whisker plots and univariable analysis of the association between each of the mammographic parameters and the range of potential determinants.

Characteristics of study participants were summarised for treated and untreated women and p-values for differences in characteristics were calculated using t-tests for means and chi-square for proportions. Following convention, nominal statistical significance was based on a p-value of less than 0.05. All tests of significance were two-sided. (See Section 4.2.6 for a discussion on the choice of threshold for statistical significance.)

7.2.8.2 Regression analysis

Multiple linear regression was then used to assess effects of treatment (regression coefficient representing the difference between means of treated and untreated women after adjustment for covariates).

Least square means

The estimate for treatment effect was difficult to interpret because of the transformed nature of the dependent variables. Least squares means (LSMs) for each treatment were calculated from the regression coefficient estimates adjusted for mean age and BMI and number of livebirths. For ease of interpretation, these LSMs and their confidence intervals were back-transformed.

Analysing potential confounders

Potential confounders were entered into regression models that included treatment, starting with age and BMI. Thereafter, order was determined by forward selection, and variables were retained if the treatment coefficient changed by 10% or more.

Interactions were examined by age (before and after 50 years) and treatment type because a greater proportion of women 50 years or older were treated with DES. The difference in each of the dependent variables between treated and untreated women was examined by treatment type using a variable coded as 0 for untreated, 1 for EE and 2 for DES. Two women were treated with both and were not included in this sub-analysis.

Post-estimation diagnostics

Post-estimation diagnostic tests were performed to evaluate the validity of the regression results and included tests of collinearity and normality including the functional form of the model; residual vs predictor plots, tests of outliers and leverage, and Cook's distance.

Component plus residual plots indicated non-linearity of the BMI variable against non-dense area of the breast. A fractional polynomial model comparison test was performed and the square root inverse model was found to be the best fit. BMI was inverse square root transformed in the regression analysis examining treatment effect on non-dense area.

Sub-group analysis

The association between treatment duration and effectiveness, and age and pubertal stage at start of treatment with each of the dependent variables was examined within the treated sub-group only.

In order to investigate whether there was evidence of selective participation at follow-up 2, the characteristics of participants were compared with those of participants at follow-up 1 with respect to age, height, BMI and history of having had a breast biopsy.

Sensitivity analysis

Twenty-one of the images (13 treated, 8 untreated) were derived from digital images. A repeat analysis was performed with these removed.

Women with a history of breast cancer may have been more likely to participate in the study, or depending on the stage of their illness, less likely. More treated than untreated participants had a history of breast cancer. It is possible that these cases may have inflated the difference in dense area observed between treated and untreated women. Participants who had a history of breast cancer were removed and a repeat analysis performed.

As an additional measure, women who self-referred themselves to the study were removed from the analysis. The results of the restricted analysis were similarly presented.

7.3 Results

7.3.1 Characteristics of study participants

Age, reproductive, anthropometric, lifestyle and reproductive disease characteristics of study participants are presented separately in **Tables 7.1** to **7.5** and described below.

7.3.1.1 Age and reproductive characteristics of participants

Table 7.1 describes the age and reproductive characteristics of both treated and untreated women. Treated women were slightly older at time of interview and at mammogram than untreated women and were more likely to be postmenopausal (using either definition of menopausal status), but were similar in age at menarche, mean age at first livebirth, and total duration of breastfeeding in those who had ever breastfed. As reported elsewhere[25], treated women were less likely (78.4%) to have ever had a livebirth than untreated women (81.7%), though this difference was small (p=0.48).

Table 7.1: Age and reproductive characteristics of study participants.

Characteristic	Treated (n=167)	Untreated (n=142)	P-value
Age Mean (SD) (years)			
At interview	48.4 (4.8)	46.2 (4.1)	**<0.001**
At mammogram	48.0 (4.7)	45.8 (4.1)	**<0.001**
Postmenopausal n (%) *	57 (34.1)	25 (17.6)	**0.001**
Postmenopausal n (%) †	23 (13.8)	8 (5.6)	**0.018**
Age at menarche ‡ Mean (SD) (years)	12.8 (1.6)	12.8 (1.4)	0.858
Livebirths n (%)			
0	36 (21.6)	26 (18.3)	0.478
1	23 (13.8)	11 (7.8)	
≥2	108 (64.7)	105 (74)	

Characteristic	Treated (n=167)	Untreated (n=142)	P-value
Age at first livebirth Mean (SD) (years)	30.0 (4.6)	29.4 (4.5)	0.248
Ever breastfeed n (%)	125 (75.8)	115 (81)	0.197
Breastfeeding total mean (range) (weeks) §	98.7 (2–374)	97.2 (0.7–309.6)	0.848

* Definition of postmenopausal: last period ≥52 weeks, and if HRT started before last period and current age was ≥55 years.

† Definition of postmenopausal: Same as above but women who had not had a period for ≥52 weeks because of hysterectomy (while retaining one or both ovaries), endometrial ablation, IUD, or hormone implants, they were considered to be premenopausal unless they were ≥55 years of age.

‡ Age at menarche: 1 missing

§ Breastfeeding duration of women who had ever breastfed and for all livebirths.

7.3.1.2 Anthropometric characteristics of participants

Table 7.2 describes the anthropometric characteristics of treated and untreated women. Treated women were slightly taller and had a lower BMI than untreated women. Untreated women were slightly heavier than treated women, and had a greater weight gain between the ages 18 and 30, 18 to current and 30 to current, but these differences were small (p>0.30).

Table 7.2: Anthropometric characteristics of participants.

Characteristic	Treated (n=167)	Untreated (n=142)	P-value
Height (cm)			
Mean (SD) (range)	178.4 (3.8) (165.0 to 191.8)	175.6 (4.7) (160.0 to 194.0)	**<0.001**
Weight (kg)			
Mean (SD) (range)	78.4 (16.2) (52.0 to155.0)	79.9 (15.7) (52.0 to 127.0)	0.397
BMI (kg/m^2)			
Mean (SD) (range)	24.6 (5.2) (16.9 to 49.6)	25.9 (4.9) (18.0 to 40.3)	**0.030**
EMH–final height (cm)[1]			
Mean (SD) (range)	2.1 (2.8) (–3.5 to 11.7)	–1.0 (3.2) (–12.3 to 9.0)	**<0.001**
Weight change (kg)			
Mean (SD) (range)			
18 to 30 years	4.4 (7.7) (–27.8 to 41.5)	4.6 (7.0) (–11 to 33)	0.765
18 years to current	12.3 (12.2) (–25.0 to 59.0)	13.8 (12.5) (–10.0 to 50.0)	0.314
30 years to current	7.9 (10.7) (–13.0 to 59.0)	9.1 (10.0) (–24.0 to 56.5)	0.304

A summary of childhood anthropometric characteristics by treatment status, is presented and described in Chapter 8 (**Table 8.1**). These variables include birthweight, birth-length, bone age minus chronological age, and height, weight and BMI at first assessment. As well, weight and BMI change the first year following treatment, age at maximum height, and height change after 15 years is described for treated women only. The analyses of these data are described in Chapter 8 also (**Table 8.2**).

7.3.1.3 Use of hormone and related medicines by participants

Table 7.3 describes the use of hormones and hormone related medicines in treated and untreated women. Treated women were more likely to have had fertility drug treatment (consistent with fertility effects reported elsewhere[25]) and to currently use, and have ever used hormone replacement therapy, particularly the estrogen only formulation, but were similar in oral contraceptive use (ever used and currently use), though treated women used oral contraceptives for a longer duration.

Table 7.3: Use of hormone or related medications by treatment status.

Characteristic	Treated (N=167)	Untreated (N=142)	P-value
Fertility drugs taken n (%)	43 (25.8)	18 (12.7)	**0.004**
Fertility cycles Mean (range)	6.0 (1 to 18)	7.2 (1 to 36)	**0.117**
Fertility cycles (categories)			
0	124 (74.3)	124 (87.3)	**0.004**
1–<5	18 (10.8)	9 (6.3)	0.168
5–<10	16 (9.6)	4 (2.8)	**0.016**
≥10	9 (2.1)	5 (3.5)	0.431
HRT			
Ever used n (%)	35 (21.0)	15 (10.6)	**0.013**
Current use	22 (13.2)	7 (4.9)	**0.013**
Total use (years)			
Mean (range)*	3.9 (0.02 to 27.7)	2.6 (0.02 to 10.6)	0.073
HRT type n (%) †			
Estrogen	17 (10.2)	4 (2.8)	**0.010**
Estrogen & progestagen	12 (7.2)	8 (5.6)	0.581
Progestagen	4 (2.4)	2 (1.4)	0.531
Testosterone, estrogen & progestagen	0	1 (0.7)	0.277
Unknown	2 (1.2)	0	

Characteristic	Treated (N=167)	Untreated (N=142)	P-value
Hormonal contraceptive			
Ever used n (%)	161 (96.4)	138 (97.2)	0.701
Current use n (%)	20 (12.0)	22 (15.5)	0.369
Total use (years)‡			
Mean (range)	10.1 (0.08–33)	11.9 (0.11–29.2)	**0.028**
Age first used			
Mean (range)	19.4 (13–36)	19.1 (13–33)	0.495
Ever used hormones for endometriosis n (%)	10 (6.0)	4 (2.8)	0.182
Duration of hormone use to treat endometriosis (weeks)			
Median (95% CI)	26 (13–192)	26 (26–104)	0.452
Ever used hormones for menstrual problems n (%)	9 (5.4)	10 (7.0)	0.547
Ever used aspirin § n (%)	22 (13.2)	18 (12.7)	0.897
Ever used over the counter anti-inflammatories § n (%)	21 (12.6)	11 (7.8)	0.165
Ever used prescription anti-inflammatories § n (%)	37 (22.2)	28 (19.9)	0.622

* HRT total use for treated n=1 missing

†% is of total treated and untreated, not just those who used HRT

‡ Of those who used hormonal contraceptive

§ Twice a week for a month or longer

7.3.1.4 Smoking, alcohol use and socio-demographic characteristics of participants

Table 7.4 describes the smoking, alcohol use and socio-demographic characteristics of treated and untreated women. Treated women had a similar smoking and alcohol history than untreated women. The majority of treated (94.0%) and untreated women (98.6%) were born in Australia and marital status and educational levels were similar for both groups.

Table 7.4: Smoking, alcohol and socio-demographic characteristics of participants by treatment status.

Characteristic	Treated n (%) (N=167)	Untreated n (%) (N=142)	P-value
Smoking			
Ever smoked	88 (52.7)	83 (58.4)	0.310
Currently smoke	18 (10.8)	17 (12.0)	0.741
Alcohol use			
Never or rarely drink	24 (14.3)	24 (16.9)	0.541
Occasionally (<once a week)	28 (16.8)	25 (17.6)	0.845
Once or twice a week	33 (19.8)	34 (23.9)	0.374
Three or more days a week	82 (49.1)	59 (41.5)	0.184
Marital status			
Married	115 (68.9)	96 (67.6)	0.813
De facto	15 (9.0)	11 (7.8)	0.697
Separated	6 (3.6)	5 (3.5)	0.973
Divorced	9 (5.4)	6 (4.2)	0.683
Widowed	6 (3.6)	2 (1.4)	0.228
Single	16 (9.6)	22 (15.5)	0.115
Educational level			
Primary School	0	0	-
Intermediate/Year 11	5 (3.0)	7 (4.9)	0.380
High School/Year 11 & 12	33 (19.8)	22 (15.5)	0.328
Certificate/Diploma	42 (25.2)	34 (23.9)	0.806
University Degree	49 (29.3)	50 (35.2)	0.270
Higher University Degree	38 (22.8)	29 (20.4)	0.620
Country of Birth			
Australia	157 (94.0)	140 (98.6)	**0.038**
UK	7 (4.2)	1 (0.7)	-
Other	3 (1.8)	1 (0.7)	-

7.3.1.5 History of reproductive disease in participants

Table 7.5 describes the reproductive disease history of treated and untreated women and breast and ovarian cancer in their first degree relatives. Treated women were no more likely to have been diagnosed with benign breast disease and endometriosis compared with untreated women. Ovarian cysts were a reported short-term side effect of treatment in some treated girls in this cohort (data not presented), and in the literature[29], possibly explaining the difference observed in the history of having had ovarian cysts between treated and untreated women.

Table 7.5: History of reproductive disease in treated and untreated women.

Characteristic	Treated n (%) (N=167)	Untreated n (%) (N=142)	P-value
Benign breast disease	37 (22.2)	23 (16.2)	0.187
Polycystic ovary syndrome	5 (3.0)	4 (2.8)	0.926
Ovarian cysts	39 (23.4)	20 (14.1)	**0.04**
Uterine fibroid	28 (16.8)	24 (16.9)	0.975
Endometriosis	30 (18.0)	16 (11.3)	0.100
Breast cancer	1 (0.6)	3 (2.1)	0.241
Vaginal/Uterine cancer	5 (3.0)	6 (4.2)	0.560
Breast cancer: 1st degree relative	27 (16.2)	19 (13.4)	0.493
Ovarian cancer: 1st degree relative	2 (1.2)	3 (2.1)	-

7.3.1.6 Characteristics of eligible participants and non-participants

Characteristics were compared between eligible women who participated in the mammographic density study (n=309) and eligible women who did not (n=208). Participants were of similar age (mean 47.5 years), height (mean 177.4 cm) and BMI (mean 24.7 kg/m^2) to non-participants (mean age=46.3 years; mean height=177.5 cm; mean BMI 24.7 kg/m^2). History of having had a breast biopsy was also similar for participants (12.9%) and non-participants (13.9%).

7.3.2 Exposure characteristics

A description of treatment duration, type and timing in relation to pubertal development is presented in **Table 7.6**. Of the 167 treated women participating in the study, more women were treated with DES, than were treated with EE. Treatment type was unknown for 13 (7.8%) women. The mean age at start of treatment was 12.8 (SD 1.7) years, with a mean treatment duration of 23.6 months (21.0 months EE, 25.8 months DES). Treatment commenced after menarche in 85 (51.2%) women. Data were available on Tanner Stage of breast development for 103 (61.7%) treated women. Of these, 82.5% commenced treatment at or after Tanner Stage 3.

Table 7.6: Treatment characteristics (treated women only).

Characteristic	Treated (all)	EE	DES
Treatment type* n (%)	167	62 (37.1)	90 (53.9)
Age, start of treatment			
Mean (range)	12.8 (8–17.5)	13.2 (8–17.5)	12.7 (9.0–16.2)
Duration of Treatment†			
Mean (range)(months)‡	24.3 (1.8–96)	24.1 (1.8–96)	24.6 (4.3–49.1)
Mean (range) (years) §	1.97 (0.2–4.1)	1.75 (0.2–3.3)	2.15 (0.4–4.1)
Treatment commence ¶			
Before menarche n (%)	81 (48.8)	29 (46.8)	44 (49.4)
After menarche n (%)	85 (51.2)	33 (53.2)	45 (50.6)
Breast Tanner Stage at beginning of treatment n (%)			
1	3 (1.8)	0 (0)	3 (3.3)
2	15 (9.0)	5 (8.1)	10 (11.1)
3	40 (24.0)	15 (24.2)	24 (26.7)
4	23 (13.8)	8 (12.9)	15 (16.7)
5	22 (13.2)	14 (22.6)	8 (8.9)
Missing	64 (38.3)	20 (32.3)	30 (33.3)

* Number of treated who used both types at different times n=2 (1.2%); number of treated whose treatment type is missing n=13 (7.8%)

†Missing n=3

‡ Sourced from medical records and self report

§ Source from Medical records only (n=111)

¶ Missing n=1

7.3.3 Mammographic density characteristics of participants

While dense area and percent density are the primary outcome measures of interest to this study, both non-dense area and total breast area will be described and similarly analysed as they may explain and confirm the differences, if any, observed between dense and percent density.

Mean and median values of the mammographic density parameters dense area, percent density, total breast area and non-dense area are presented in **Table 7.7** for treated and untreated women. Mean dense area was less in treated women than untreated women. There was no difference in percent density and non-dense area between treated and untreated women. Total breast area was greater in untreated women compared to treated women but this difference was not statistically significant.

Table 7.7: Mean and median values of mammographic density parameters: dense area (cm^2), percent density (%), total breast area (cm^2) and non-dense area (cm^2).

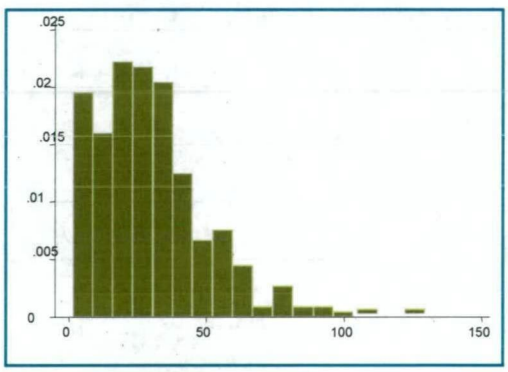
Characteristic	Treated	Untreated	P-value
Dense area (cm^2)			
Median (5^{th} & 95^{th} percentile)	26.4 (3.1–60.0)	27.8 (4.7–77.6)	-
Mean (SD)	27.6 (17.6)	32.8 (22.8)	**0.022**
Percent density (%)			
Median (5^{th} & 95^{th} percentile)	26.8 (2.2–67.1)	28.2 (2.5–66.1)	-
Mean (SD)	29.5 (20.4)	30.8 (19.6)	0.575
Total breast area (cm^2)			
Median (5^{th} & 95^{th} percentile)	102.6 (49.7–232.9)	111.8 (57.4–236.0)	-
Mean (SD)	114.5 (57.2)	123.9 (55.5)	0.149
Non-dense area (cm^2)			
Median (5^{th} & 95^{th} percentile)	71.7 (18.5–226.9)	69.2 (27.0–225.8)	-
Mean (SD)	87.0 (60.5)	91.1 (59.8)	0.554

7.3.4 Transformation of outcome variables

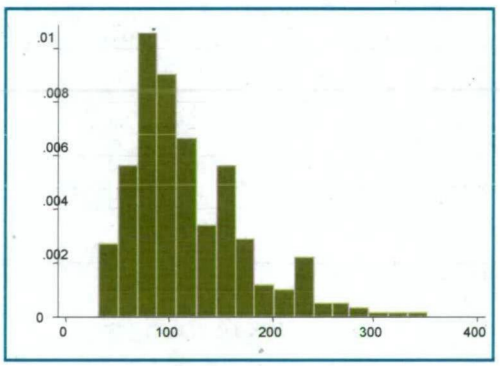
Dense area and percent dense area were square root transformed while total breast area and non-dense areas were log transformed to fulfill the assumption of normality for regression analyses. The distributions of the raw and transformed data for each of these mammographic parameters are plotted in histograms in **Figures 7.5** and **7.6** respectively.

Figure 7.5: Distribution of dense area, percent density, total breast area, non-dense area.

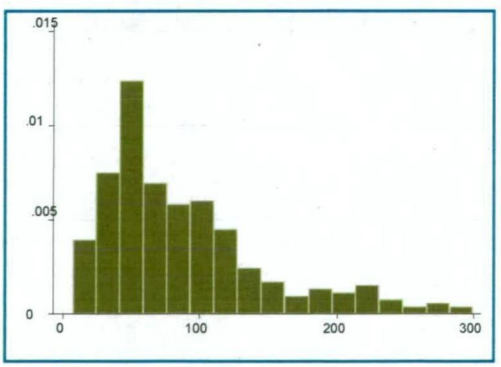
Absolute density (cm^2)



Total area (cm^2)



Non-dense area (cm^2)



Percent density (%)

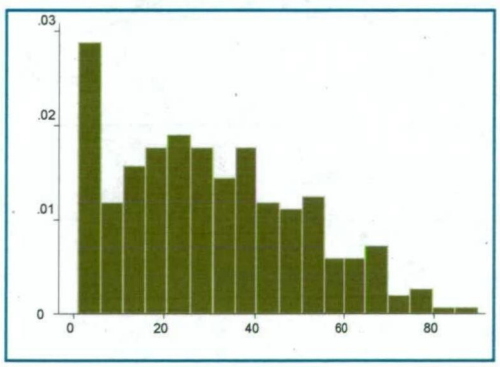
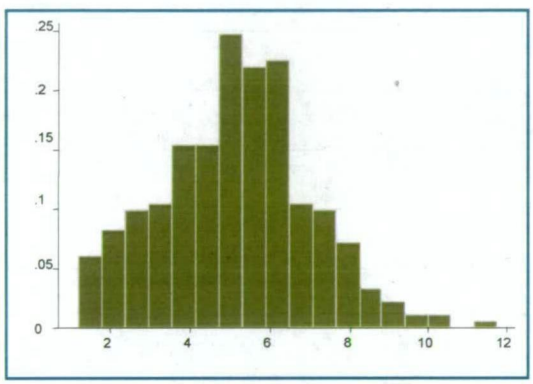
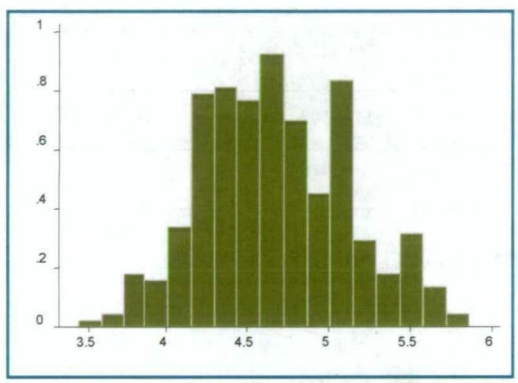


Figure 7.6: Distribution of square root transformed dense area and percent density, and log transformed total breast area and non-dense area.

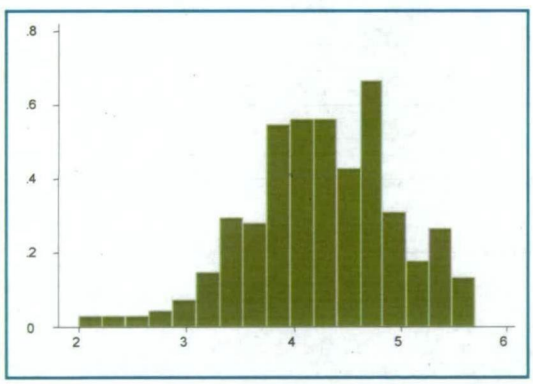
Square root dense area (cm^2)



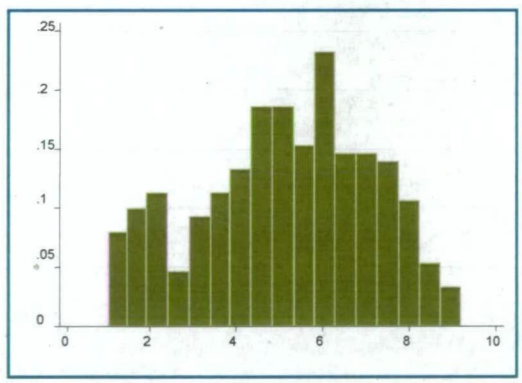
Log total breast area (cm^2)



Log non-dense area (cm^2)



Square-root percent density (%)



7.3.5 Univariable analysis

A univariable analysis of the association between treatment and each of the mammographic measures: dense area (sqrt), percent density (sqrt), non-dense area (log) and total breast area (log) is described below. This is followed by a univariable analysis of single covariates and each of the mammographic measures.

7.3.5.1 Univariable analysis of association between treatment and mammographic outcome variables

The results of a univariable analysis of the association between the binary treatment variable (treated=1, untreated=0) and the transformed dependent outcome variables are presented in **Table 7.8**. Dense area and total breast area were significantly associated with treatment status. The direction of the coefficients indicates that treated women have a lower mean dense area and total breast area than untreated women.

Table 7.8: Univariable analysis of the association between treatment status and the outcome variables: dense area cm^2 (sqrt), percent density (%), total breast area cm^2 (log) and non-dense area cm^2 (log).

Independent variable	Dependent variable	Coefficient (95% CI)	P-value
Treatment status	Dense area	–0.45 (–0.87 to –0.04)	**p=0.03**
	Percent density	–0.17 (–0.62 to 0.28)	p=0.47
	Total breast area	–0.09 (–0.19 to 0.01)	**p=0.07**
	Non-dense area	–0.08 (–0.23 to 0.08)	p=0.32

7.3.5.2 Univariable analysis of the association between single covariates and the mammographic outcome variable

A number of potential confounding or mediating variables were examined for a univariable association with the outcome variables: sqrt transformed dense area (cm^2), sqrt percent density (%), log transformed total breast area (cm^2) and log non-dense area (cm^2). T-tests were used for continuous variables and chi-squared tests for binary variables. Those variables for which an association was observed for any one of the four mammographic variables (p-value > 0.10) are highlighted in bold in **Table 7.9** below.

Table 7.9: Univariable analysis of the association between potential influencing factors and the outcome variables dense area (cm^2) (sqrt), percent density (%), total breast area (cm^2) (log) and non-dense area (cm^2) (log).

Variable	Dense Area (sqrt) Coefficient (95% CI)	P-value	% Density (sqrt) Coefficient (95% CI)	P-value	Total Breast Area (log) Coefficient (95% CI)	P-value	Non-dense Area (log) Coefficient (95% CI)	P-value
Age	–0.08 (–0.12 to –0.04)	**0.001**	–0.10 (–0.14 to –0.05)	**<0.001**	0.01 (–0.003 to 0.02)	0.171	0.03 (0.01 to 0.04)	**0.002**
Menopause[1]	–0.62 (–1.09 to –0.15)	**0.009**	–0.77 (–1.27 to –0.26)	**0.003**	0.08 (–0.03 to 0.20)	0.159	0.21 (0.04 to 0.38)	**0.018**
Menopause[2]	–1.22 (–1.90 to –0.54)	**<0.001**	–1.38 (–2.11 to –0.65)	**<0.001**	0.10 (–0.07 to 0.27)	0.233	0.31 (0.05 to 0.57)	0.018
Age at menarche	0.17 (0.04 to 0.31)	**0.012**	0.37 (0.23 to 0.52)	**<0.001**	–0.10 (–0.13 to –0.06)	**<0.001**	–0.15 (–0.20 to –0.10)	**<0.001**
BMI	–0.10 (–0.14 to –0.06)	**<0.001**	–0.21 (–0.25 to –0.17)	**<0.001**	0.06 (–0.05 to 0.06)	**<0.001**	0.09 (0.08 to 0.10)	**<0.001**
Height	0.01 (–0.03 to –0.06)	0.579	0.03 (–0.01 to 0.09)	0.164	–0.01 (–0.02 to 0.002)	0.123	–0.02 (–0.04 to –0.001)	**0.040**
Weight	–0.03 (–0.04 to –0.02)	**< 0.001**	–0.07 (–0.08 to –0.05)	**< 0.001**	0.02 (0.02 to 0.02)	**< 0.001**	0.03 (0.02 to 0.03)	**< 0.001**
Ever used HRT	–0.63 (–1.19 to –0.07)	**0.028**	–0.63 (–1.24 to –0.02)	**0.042**	–0.04 (–0.18 to 0.10)	0.562	0.08 (–0.13 to 0.29)	0.435
Benign breast disease	0.56 (0.04 to 1.08)	**0.036**	0.43 (–0.14 to 0.99)	0.141	0.02 (–0.10 to 0.15)	0.709	–0.05 (–0.25 to 0.14)	0.603

Variable	Dense Area (sqrt) Coefficient (95% CI)	P-value	% Density (sqrt) Coefficient (95% CI)	P-value	Total Breast Area (log) Coefficient (95% CI)	P-value	Non-dense Area (log) Coefficient (95% CI)	P-value
Ovarian cysts	–0.34 (–0.87 to 0.19)	0.210	–0.72 (–1.3 to –0.15)	**0.013**	0.18 (0.05 to 0.30)	**0.006**	0.29 (0.10 to 0.48)	**0.004**
Endometriosis	0.71 (0.13 to 1.29)	**0.017**	0.51 (–0.12 to 1.14)	0.113	0.001 (–0.14 to 0.14)	0.988	–0.04 (–0.26 to 0.17)	0.686
Ever smoked	0.13 (–0.28 to 0.55)	0.521	0.02 (–0.43 to 0.47)	0.931	0.05 (–0.05 to 0.15)	0.309	0.05 (–0.11 to 0.20)	0.555
Currently smoke	–0.89 (–1.54 to –0.24)	**0.007**	–1.08 (–1.78 to –0.38)	**0.003**	0.14 (–0.02 to 0.30)	**0.086**	0.31 (0.07 to 0.55)	**0.012**
Alcohol (never/rarely)	–0.56 (–1.13 to 0.01)	**0.054**	–0.72 (–1.34 to –1.09)	**0.021**	0.09 (–0.05 to 0.23)	0.221	0.19 (–0.02 to 0.40)	**0.080**

* Definition of postmenopausal: last period ≥ 52wks, and if HRT started before last period and current age was ≥55 years.

† Definition of postmenopausal: Same as above but women who had not had a period for ≥52 weeks because of hysterectomy (while retaining one or both ovaries), endometrial ablation, IUD, or hormone implants, they were considered to be premenopausal unless they were ≥55 years of age

The univariable analyses demonstrated a number of associations with one or more of the mammographic outcome variables. The following variables were inversely and significantly associated with dense area: age, menopause[1, 2], weight, BMI, HRT (ever used) and smoking (current). In contrast, age at menarche, diagnosis of benign breast disease or endometriosis, and alcohol use were positively associated with dense area. Since dense area is strongly associated with age, this variable was added to the regression. All of these associations remained after adjustment for current age except for menopause[1] and ever use of HRT.

Similar associations were observed for percent mammographic density as was observed for dense area, apart from diagnosis of benign breast disease or endometriosis which were not significantly associated with percent density. Ever been diagnosed with an ovarian cyst was significantly and inversely associated with percent density. All of these associations remained after adjustment for current age, except for menopause[1] and ever use of HRT.

Total breast area was negatively and significantly associated with age at menarche, and positively associated with BMI, weight and having been diagnosed with an ovarian cyst. These associations remained after adjustment for current age.

Non-dense area was positively and significantly associated with age, menopause[1,2], BMI, weight, smoking (current) and ever been diagnosed with having had an ovarian cyst. It was negatively associated with age at menarche and height. The association with menopause[1,2] and height did not remain after adjustment for current age.

Variables that were not significantly associated with any one of the four mammographic outcome variables included: number of livebirths, age at first livebirth, breastfeeding, current use of HRT, ever or currently using hormonal contraceptives, fertility drug use (ever), and first degree family member with breast cancer (See **Table 7.10**).

Table 7.10: Univariable analysis of the association between potential influential variables and the outcome variables dense area (cm^2) (sqrt), percent density (%) (sqrt), total breast area (cm^2) (log) and non-dense area (cm^2) (log).

Variable	Dense Area (sqrt) Coefficient (95% CI)	P-value	% Density (sqrt) Coefficient (95% CI)	P-value	Total Breast Area (log) Coefficient (95% CI)	P-value	Non-dense Area (log) Coefficient (95% CI)	P-value
Livebirths	–0.09 (–0.27 to 0.09)	0.324	–0.09 (–0.30 to 0.10)	0.323	–0.01 (–0.04 to 0.05)	0.828	0.03 (–0.04 to 0.09)	0.462
Age at first livebirth	0.04 (–0.01 to 0.09)	0.110	0.03 (–0.02, to 0.09)	0.266	0.0003 (–0.01 to 0.01)	0.966	–0.002 (–0.02 to 0.02)	0.780
Breastfeeding (total weeks)	0.001 (–0.002 to –0.004)	0.689	0.001 (–0.002 to 0.004)	0.622	–0.0001 (–0.001 to 0.001)	0.767	–0.0002 (–0.001 to 0.001)	0.722
HRT Current	–0.30 (–1.0 to 0.42)	0.416	–0.15 (–0.93 to 0.62)	0.694	–0.08 (–0.26 to 0.09)	0.349	–0.05 (–0.31 to 0.22)	0.736
OC use current	–0.02 (–0.63 to 0.59)	0.954	–0.12 (–0.78 to 0.54)	0.718	0.04 (–0.11 to 0.19)	0.594	0.09 (–0.14 to 0.31)	0.451
OC use ever	0.47 (–0.70 to 1.65)	0.428	0.56 (–0.71 to 1.83)	0.383	–0.04 (–0.32 to 0.25)	0.789	–0.07 (–0.51 to 0.37)	0.758
Fertility drug (ever used)	–0.03 (–0.56 to 0.49)	0.898	–0.12 (–0.69 to 0.54)	0.664	0.02 (–0.11 to 0.14)	0.812	0.06 (–0.14 to 0.25)	0.565

7.3.6 Box-plots of mammographic measures

Box-plots are illustrated in **Figures 7.7** and **7.8** below. The mammographic outcome measures are on the y-axis and the covariate of interest on the x-axis of each plot. The Box-plots represent the median (mid-line in coloured boxes) the top and bottom of the box represents the 25th and 75th percentiles. Since these plots represent the median, untransformed outcome data was used.

Menopausal status and BMI are examined in these Box-plots for illustrative purposes. A range of other variables were also examined but are not included. Dense area and percent density appear to be lower in older and postmenopausal women (**Figure 7.7**), consistent with the findings of the univariable analyses in **Table 7.9** above. The opposite can be seen for total breast area and non-dense area (**Figure 7.8**). Median total breast area and median non-dense area appears to be greater in older and postmenopausal women. However, the plots suggest that menopause has a more dramatic effect in women before 50 years of age compared to women older than 50. It could be that the effect of age reduced baseline dense area or percent density for premenopausal women after 50 compared to <50 years, and consequently reduced the potential for further reduction with menopause (or increase for non-dense and total breast areas). The potential for interaction between age and menopause on each of the mammographic parameters is examined later. Similar box plots are presented for the alternative definition of menopause in Appendix 16.

Figure 7.7: Box-plots of dense area and percent density by menopausal status and age category (<50 years, ≥50years) for treated and untreated combined.

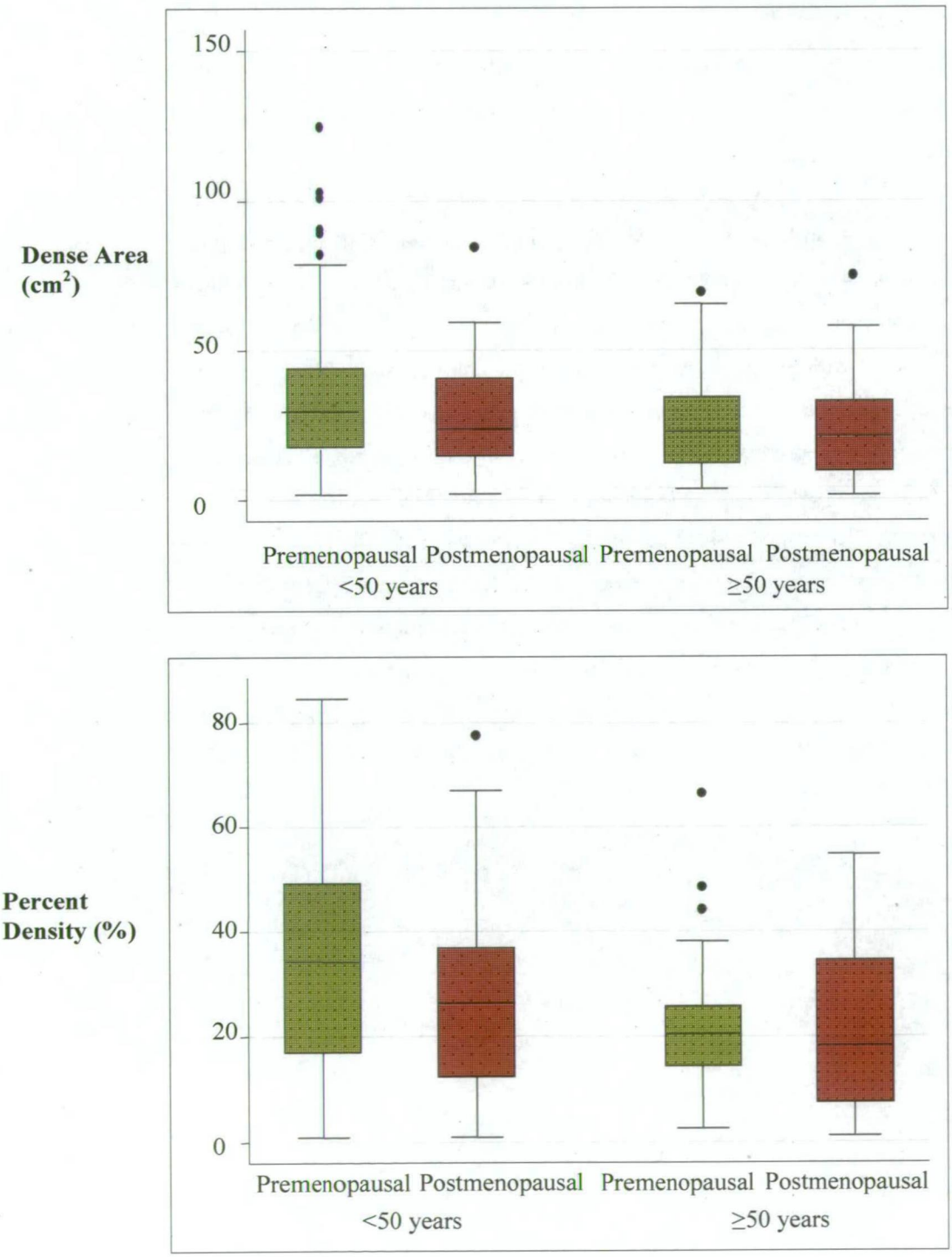
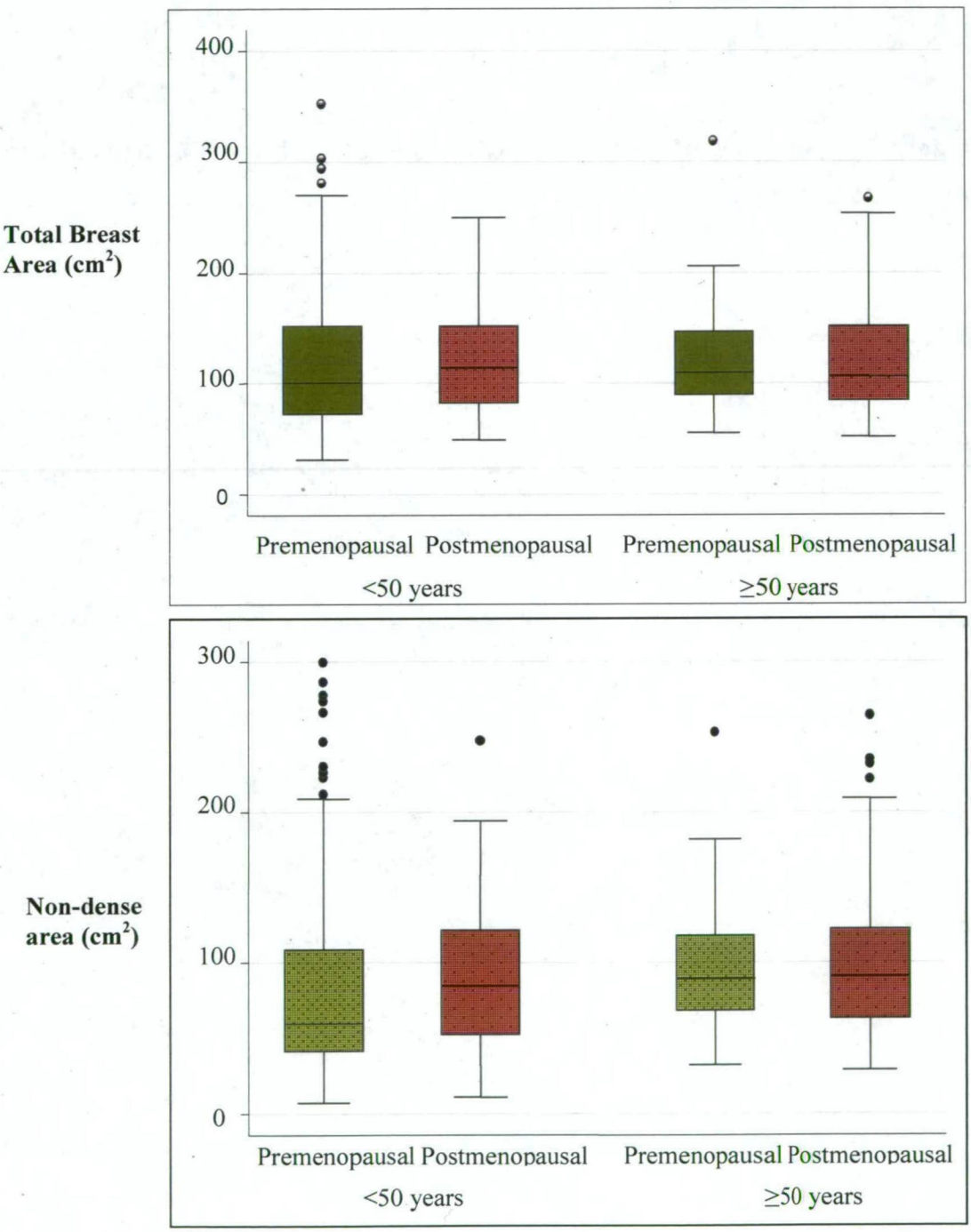


Figure 7.8: Total breast area (cm^2) and non-dense area (cm^2) by menopausal status and age category (<50 years, ≥50years) for treated and untreated combined.



BMI is negatively associated with dense area and percent density, and positively associated with total breast area and non-dense area. This is confirmed by the direction of the univariable regression estimates presented above in **Table 7.9** and the box-plots in **Figures 7.9 to 7.12** below.

Figure 7.9: Box-plot of dense area (cm^2) and BMI kg/m^2 for treated and untreated combined.

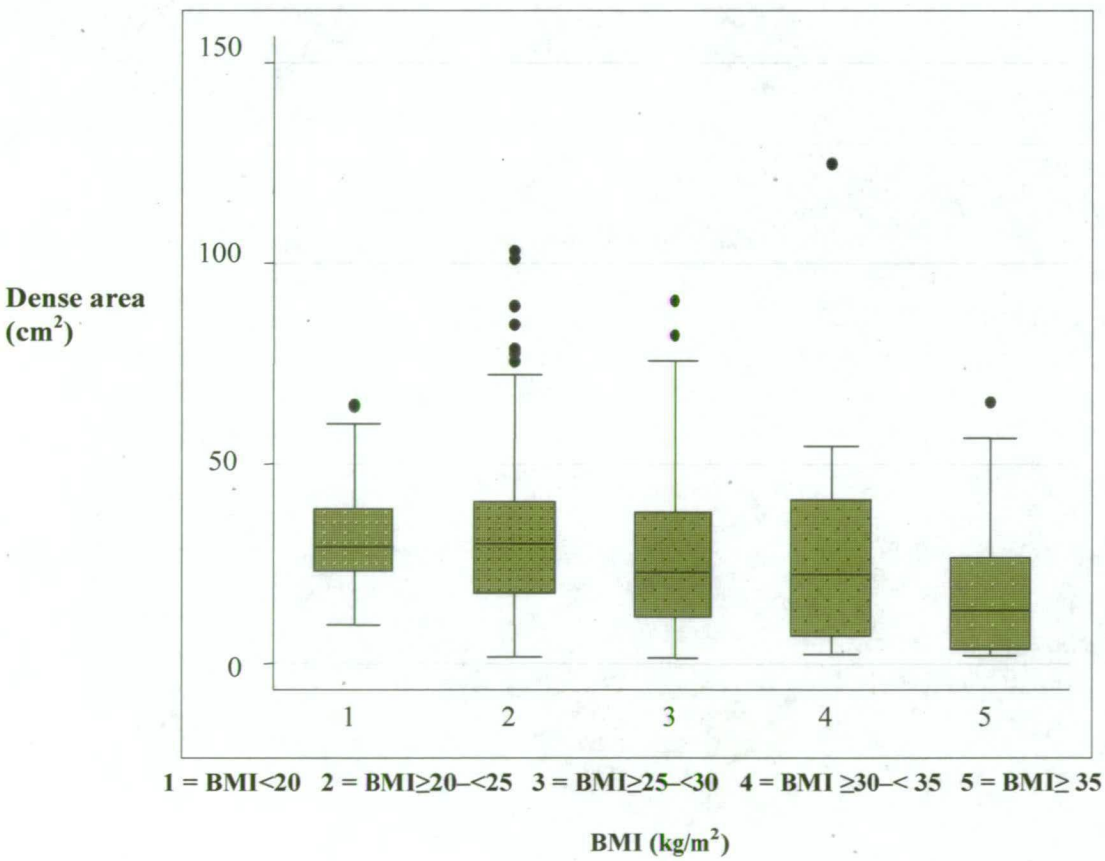


Figure 7.10: Box-plot of percent density (%) and BMI kg/m^2 for treated and untreated combined.

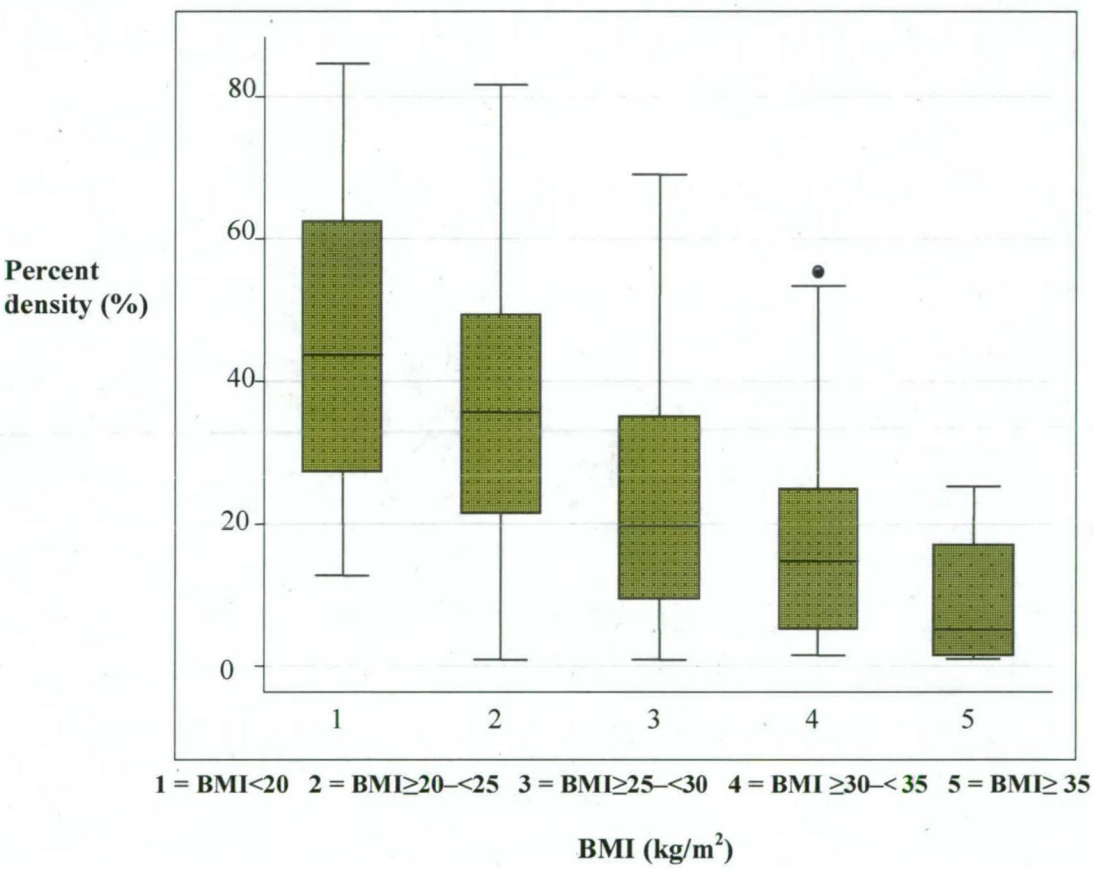


Figure 7.11: Box-plot of total breast area (log) and BMI kg/m^2 for treated and untreated combined.

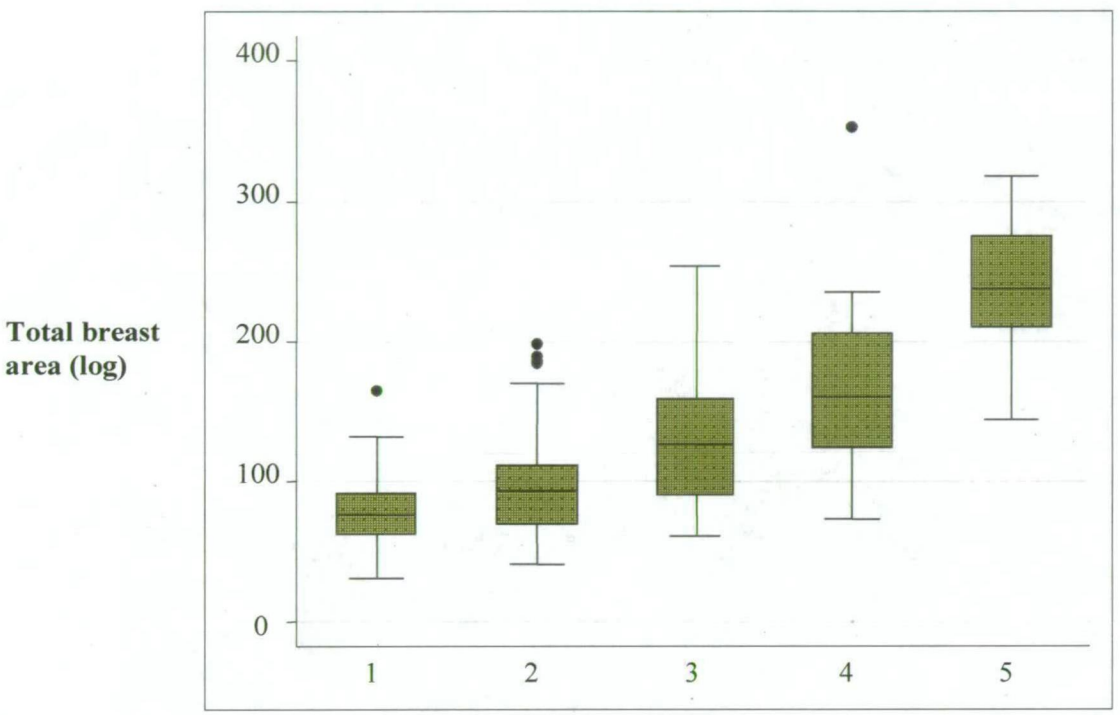
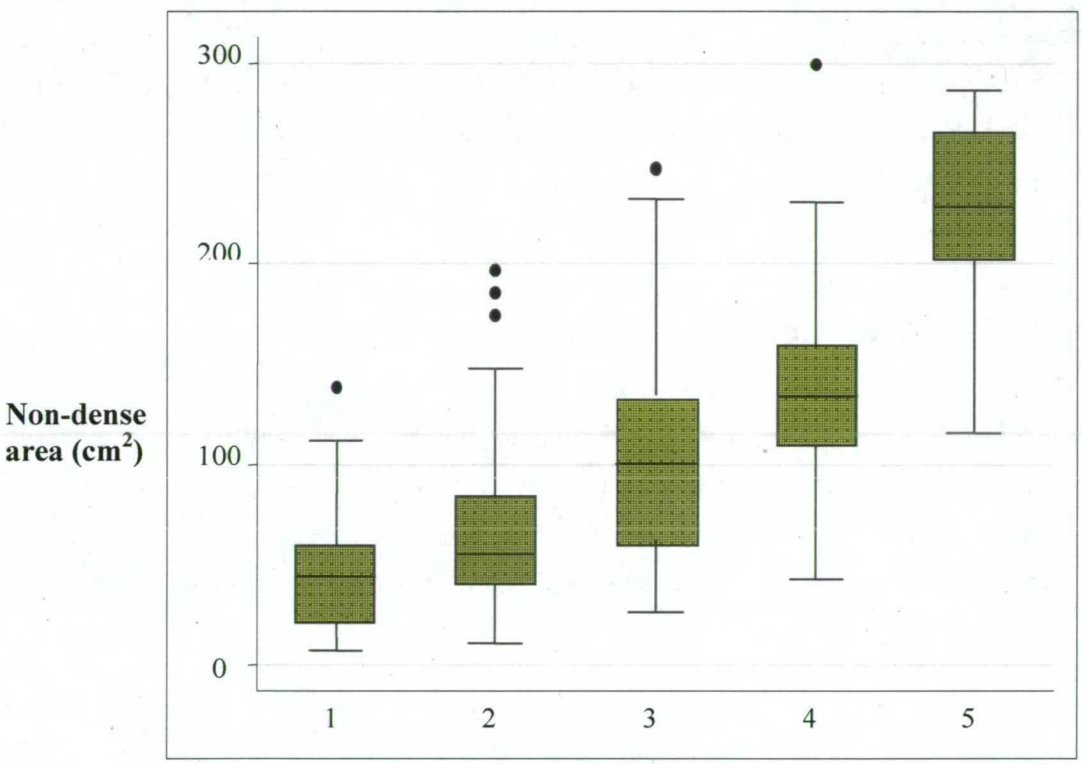


Figure 7.12: Box-plot of non-dense area (log) and BMI kg/m2 for treated and untreated women combined.



7.3.7 Multivariable analysis

Potential anthropometric, reproductive, hormonal and lifestyle determinants described in **Tables 7.1** to **7.5** [e.g. menopausal status, HRT use (ever, current, years in total), number of livebirths, age at first livebirth, age at menarche, family history of breast cancer, smoking and alcohol use, hormonal contraceptive use, and fertility hormone use], were included in the regression model for treatment effect on dense area, percent density, non-dense area and total breast area, one at a time. They were only retained if the magnitude of the coefficient for treatment changed by more than 10% with their addition to the model. If they did not fulfill this criterion, they were excluded from the analysis. The results of this analysis are presented for each of the mammographic measures separately below.

7.3.7.1 Multivariable analysis: dense area

Table 7.11 presents the regression coefficients for the treatment effect on absolute breast density (cm^2) (sqrt), adjusted for age and BMI. Additional covariates (listed in **Tables 7.1 to 7.5**) were added to the regression model one at a time to observe their effect on the coefficient of the treatment variable. Only age and BMI were found to significantly influence the treatment effect.

The results tabulated in **Table 7.11** suggest that dense area is lower in treated women than untreated women.

Table 7.11: Regression coefficients of univariable and multivariable analysis of the association between treatment status (treated, untreated) and dense area (cm^2) (square root transformed) (N=309).

	Regression coefficients (SE)		
Treatment	–0.45 (0.21)	–0.30 (0.21)	–0.45 (0.21)
Age	–	–0.07 (0.21)	–0.06 (0.02)
BMI	–	–	–0.10 (0.02)
P-value for Tx	**p = 0.032**	p = 0.163	**p = 0.032**
95% CI	–0.87 to –0.04	–0.72 to 0.12	–0.86 to –0.04

After the additional adjustment for endometriosis and benign breast disease, the association between treatment and dense area was strengthened [regression coefficient –0.54 (95%CI: –0.95 to –0.12; p=0.009)].

The adjusted coefficients were used to calculate least square means to aid in their interpretation. Treated women had lower adjusted mean dense area than untreated women. Mean dense area adjusted for age and BMI was 24.5 cm^2 (95% CI: 21.9 to 27.2) in treated women and 29.1 cm^2 (95% CI: 26.0 to 32.4) in untreated women. The difference, which equates to a mean difference of 4.6 cm^2 dense breast tissue was statistically significant (p=0.032).

Postestimation diagnostics: dense area

A number of diagnostic tests were performed on the regression model to verify that it fulfilled the assumptions of linearity and normality, and to identify and examine highly influential data points. The assumptions of linearity, and normality were found to be fulfilled. No influential points were found to significantly change the results if removed from the analysis (See Appendix 17).

Sensitivity analysis

Twenty-one of the images (13 treated, 8 untreated) were derived from digital images. Removing these values from the regression had little effect on the regression coefficient, changing it from –0.45 to –0.42 (see **Table 7.12**). The p-value moved to borderline statistical significance which is expected from the smaller sample size. Additional adjustment for covariates livebirths and current use of HRT increased the association to –0.47 (p=0.030).

Table 7.12: Regression coefficient (multivariable) for treatment effect on dense area (sqrt) with and without digital images and breast cancer cases.

	Coefficient (SE)	95% CI	P-value
Full sample regression (n=309)*	–0.45 (0.21)	–0.86 to –0.04	0.032
Regression w/o dig films (n=288)*	–0.42 (0.22)	–0.84 to –0.004	0.052
Regression w/o dig films (n=288)†	–0.47 (0.22)	–0.90 to –0.05	0.030
Regression w/o breast cancer cases (n=305)	–0.44 (0.21)	–0.84 to –0.03	0.034

* Adjusted for age and BMI
† Adjusted for age, BMI, number of livebirths and HRT (current use)

Women with a history of breast cancer may have been more likely to participate in the study, or depending on the stage of their illness, less likely. More treated than untreated participants had a history of breast cancer. It is possible that these cases may have inflated the difference in dense area observed between treated and untreated women. Removing participants who had a history of breast cancer did not have any meaningful effect on the regression coefficients (see **Table 7.12** above).

Self-referrals to the study: addressing a potential bias

As an additional measure, women who self-referred themselves to the study were removed from the analysis. Restricting the analysis did not significantly alter the results for dense area (coefficient –0.49 (95% CI: –0.95 to –0.02), p=0.041 (n=253).

Repeat measurement and analysis

To give added confidence to the positive findings for the inverse association between treatment for high-dose estrogens and dense area, the films were prepared and re-masked and breast density measurements undertaken by a second reader. This repeat analysis using a second reader's measurements of breast density also produced a statistically significant difference in dense area in treated compared with untreated women; (age and BMI adjusted regression coefficient –0.51; 95% CI: –1.02 to –0.0; p=0.048). Pearson coefficient of 0.90 for dense area and 0.92 for percent density.

Treatment type: dense area

Two forms of treatment were used, diethylstilbestrol (DES), and ethinyl estradiol (EE). The following two tables contain the regression coefficients for treatment type (DES vs no treatment, EE vs no treatment) on dense area (sqrt). **Table 7.13** contains the results for the adjusted and unadjusted analysis.

Table 7.13: Unadjusted and adjusted regression coefficients of dense area (cm^2) (sqrt) by treatment type: diethylstilbestrol (DES) and ethinyl estradiol (EE).

Treatment type*†	Unadjusted coefficient (95% CI)	P-value	Adjusted † coefficient (95% CI)	P-value
DES	–0.64 (–1.13 to –0.15)	p=0.010	–0.44 (–1.01 to 0.08)	0.11
EE	–0.27 (–0.82 to 0.28)	p=0.33	–0.53 (–1.06 to –0.01)	0.054

* Univariable categorical variable, 1=no treatment, 2=DES, 3=EE; 13 missing plus 2 who used both treatment types were not included in the analysis.

† Adjusted for age, BMI.

The results in **Table 7.13** suggest that ethinyl estradiol (EE) has a slightly larger negative coefficient than that for DES after adjustment for age and BMI. One possibility is that an interaction is acting between age and treatment type, as only two participants aged 50 years and older used EE (See **Table 7.14**). Women under 50 years of age (n=224) had a higher mean dense area (32.3 cm^2, SD 21.1) than women 50 years or older (n=85) (23.9 cm^2, SD 16.0).

Table 7.14: Number and percentage of women aged before and after 50 years by treatment type.

	No treatment	DES	EE	Unknown	Total
Age <50 years	116 (51.8%)	36 (16.1%)	60 (26.8%)	12 (5.4%)	224
Age ≥50 years	26 (30.6%)	54 (63.5%)	2 (2.4%)	3 (3.5%)	85

Interaction effects were examined between age (before and after 50 years) and treatment type, as treated women over 50 were more likely to have been treated with DES (n=54, 91.5%) than EE (n=2). No significant interaction was observed (p=0.32).

Examining the difference in treatment type by menopausal status is difficult because of low numbers (10 postmenopausal women treated with EE). Testing for interaction between treatment type (no treatment, DES and EE) with menopausal status, resulted in a p-value for interaction >0.10, when treatment type was examined within the treated group only. There was no difference in square root dense area between DES and EE treated women (p=0.6) adjusted for age and BMI.

Treatment duration and effectiveness: dense area

The observed treatment effect on breast density might be modified by the duration of treatment or the effectiveness of treatment on final height reduction. Associations between dense area and duration of treatment and estimated mature height minus final height were examined within the treated group. Regression coefficients are presented in **Table 7.15** and suggest a negative but statistically insignificant association between dense area and increasing duration of treatment. No significant association was observed between dense area and EMH minus final height, suggesting the effectiveness of treatment on height was not related to any treatment effect on the breast.

Table 7.15: Regression coefficients for duration of treatment on dense area (cm^2) (sqrt) in treated women after adjustment for age and BMI.

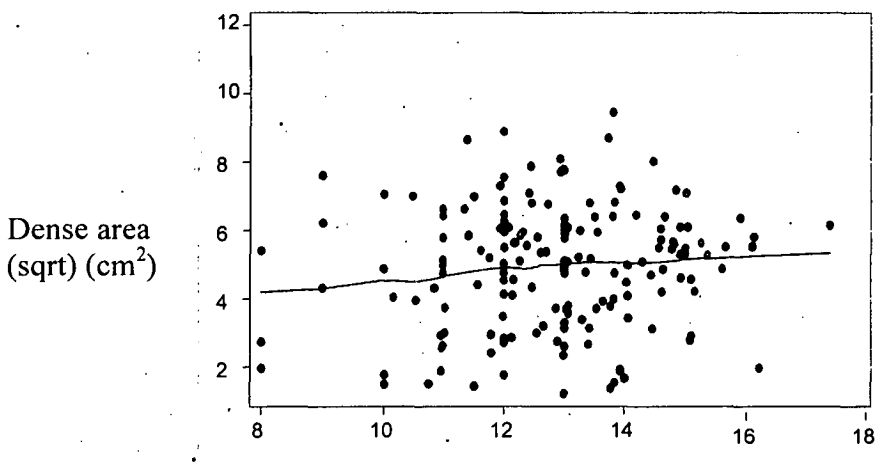
	Coefficient (SE) (95% CI)	P-value	N
Duration of treatment (years)	–0.14 (0.21) (–0.55 to 0.28)	0.51	111
EMH minus final height (cm)	0.05 (0.06) (–0.06 to 0.17)	0.38	114

* Adjusted for age and BMI

Age at start of treatment: dense area

It is possible the treatment effect on breast density is related to the timing of start of treatment. The association between dense area and age at start of treatment was examined within the treated group. Square root transformed dense area was positively associated with age at start of treatment: regression coefficient 0.12 (95% CI: –0.04 to 0.28) (p=0.154) though this association was not statistically significant. See lowess smoothed plot of dense area (y-axis) and age at start of treatment (x-axis) in **Figure.7.13**.

Figure 7.13: Smoothed lowess plot of dense area (cm^2) (sqrt) and age at start of treatment (years).



This effect did not change with adjustment for age, but disappeared after further adjustment for current BMI: coeff –0.02 (95% CI: –0.18 to 0.15) (p=0.85). These findings suggest a greater net dense area in those girls who started treatment later, or who matured later.

Treatment timing on dense area: pubertal staging

The observed treatment effect on breast density might be modified by the timing of treatment in relation to the stage of pubertal development at start of treatment. Associations between dense area and Tanner Stage of breast development and menarche at start of treatment were examined within the treated group. Dense area was greater if girls were treated after Tanner Stage 2 (breast) (age and BMI adjusted regression coefficient 0.26 (95% CI: –0.63 to 1.16) but this difference was not statistically significant (p=0.56).

There was no meaningful difference in dense area between women who were treated before menarche (median 25.2 cm^2) compared with women treated after menarche (26.4 cm^2); age and BMI adjusted regression coefficient: 0.06 (95% CI: –0.46 to 0.58) (p=0.83).

Dense area may be reduced as a result of a reduction in the total breast area, as this is observed later (though this reduction in total breast area is not significant). Adjusting for total breast area does not alter the treatment effect on dense area: regression coefficient –0.42 (SE 0.20) (95% CI: –0.82 to –0.01) (p=0.043).

Breast related side effects during treatment and dense area

Seventy-five women experienced breast related side effects (e.g. galactorrhoea, breast pain, dry and cracked nipples) (n=75) during treatment as an adolescent girl. There was no difference in dense area (sqrt) in treated women who experienced breast related side effects during treatment compared with treated women who did not (age and BMI adjusted regression coefficient 0.05 (SE 0.27) (95% CI: –0.48 to 0.57) (p=0.87).

Twelve treated women experienced breast pain as a side effect during treatment as an adolescent girl. These girls had a higher dense area (sqrt) compared to women who did not experience breast pain during treatment (age and BMI adjusted regression coefficient 0.58 (SE 0.51) (95% CI: –0.42 to 1.59) but this difference was not significant (p=0.26).

7.3.7.2 Multivariable analysis: percent mammographic density

Table 7.16 presents the adjusted regression coefficients for the treatment effect on percent mammographic density (%) (sqrt). Additional covariates (listed in **Tables 7.1** to **7.5**) were added to the regression model one at a time to observe their effect on the coefficient of the treatment variable. Only age, BMI and number of livebirths were found to influence the treatment effect. The coefficients tabulated in **Table 7.16** suggest that percent density is lower in treated women than untreated women though this difference is not statistically significant.

Table 7.16: Regression coefficients of unadjusted and adjusted analysis of the association between treatment status (treated, untreated) and percent mammographic density (%) (square root transformed) (N=309).

	Regression coefficient (SE)			
Treatment	**–0.17 (0.23)**	**–0.06 (0.23)**	**–0.25 (0.19)**	**–0.28 (0.20)**
Age (years)	–	–0.10 (0.02)	–0.08 (0.02)	–0.08 (0.02)
BMI (kg/m^2)	–	–	–0.21 (0.02)	–0.21 (0.02)
Livebirths	–	–	–	–0.10 (0.08)
P-value for Tx	0.47	0.81	0.20	0.159
95% CI	–0.62 to 0.28	–0.40 to 0.51	–0.63 to 0.13	–0.66 to 0.11

The adjusted coefficients were used to calculate least square means to aid in their interpretation. Women who had been treated with high-dose estrogens to reduce their adult height had less percent density than untreated women. Mean percent density adjusted for age, BMI and number of livebirths was 24.8% (95% CI: 22.4 to 27.4) for treated women, and 27.7% (95% CI: 24.8 to 30.7) for untreated women. This equates to a difference of 2.9% mammographic density between treated and untreated women. The difference, however, was not statistically significant. This study lacks the power to detect a significant difference of this size (see Section 7.2.2 in Methods).

Postestimation diagnostics: percent density

Post estimation diagnostics and sensitivity analysis was performed as for dense area and the assumptions of linearity, and normality were found to be fulfilled. No influential points were

found to significantly change the results. Removing digital images from the analysis did not affect the results.

Treatment type: percent density

Table 7.17 provides a summary of the results for the univariable and multivariable adjusted analysis. It appears that ethinyl estradiol has a slightly larger negative coefficient for percent mammographic density than that for DES, after adjustment for age, BMI and number of livebirths.

Table 7.17: Unadjusted and adjusted regression coefficients of percent mammographic density percent (sqrt) by treatment type: diethylstilbestrol (DES) and ethinyl estradiol (EE).

Treatment type*	Unadjusted coefficient (95% CI)	P-value	Adjusted† coefficient (95% CI)	P-value
DES	–0.45 (–0.97 to 0.07)	0.09	–0.28 (–0.78 to 0.22)	0.27
EE	0.07 (–0.82 to 0.28)	0.80	–0.40 (–0.90 to 0.10)	0.11

* Univariable categorical variable, 1=no treatment, 2=DES, 3=EE.

† Adjusted for age, BMI, number of livebirths

Treatment duration and effectiveness: percent density

The associations between percent mammographic density and duration of treatment and estimated mature height (EMH) minus final height were examined within the treated group. Regression coefficients are presented in **Table 7.18** and suggest a negative but statistically insignificant association between percent density and increasing duration of treatment. No significant association was observed between percent density and EMH minus final height.

Table 7.18: Adjusted* regression coefficients of associations between percent density (%) (sqrt) and a) duration of treatment and b) estimated mature height (EMH) minus final height (cm).

	Coefficient (SE) (95% CI)	P-value	N
Duration of treatment (years)	–0.10 (0.22) (–0.53 to 0.33)	0.64	111
EMH minus final height (cm)	0.04 (0.06) (–0.08 to 0.16)	0.52	114

* Adjusted for age, BMI and number of livebirths

Age at start of treatment: percent density

The association between percent density and age at start of treatment was examined within the treated group. Square root transformed percent density was positively and significantly associated with age at start of treatment (**Figure 7.14** and **Table 7.19**). This effect did not change with adjustment for age or livebirths but disappeared after adjusting for current BMI.

Figure 7.14: Smoothed regression and lowess curves for percent density (%) (sqrt) (y-axis) and the age at beginning of treatment (years) in treated women.

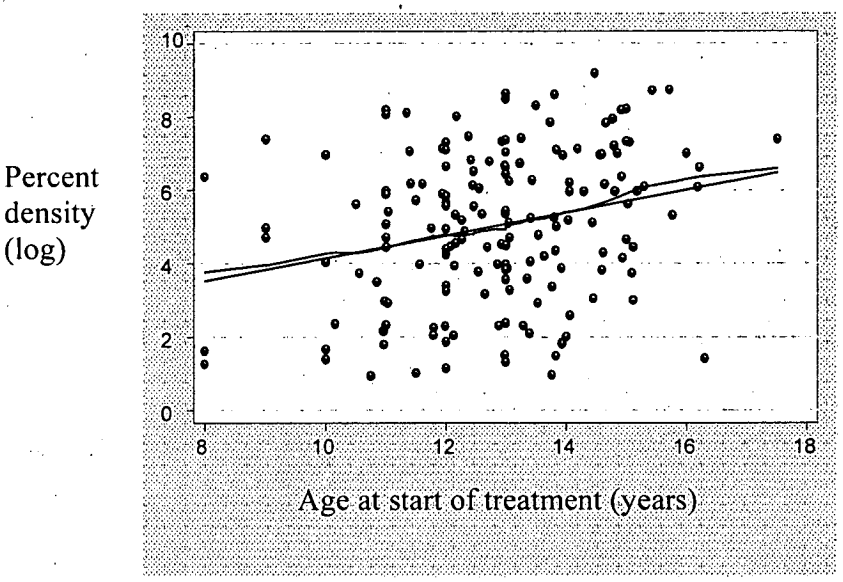


Table 7.19: Unadjusted and adjusted regression coefficients of the outcome variable percent density (%) (sqrt) and the independent variable: age at start of treatment (years).

Age at start of treatment (years)	Coefficient (SE) (95% CI)	P-value
Unadjusted	0.31 (0.001) (0.13 to 0.49)	**0.001**
Adjusted*	0.07 (0.09) (–0.10 to 0.24)	0.41

* Adjusted for age, BMI, number of livebirths (n=167)

Treatment timing on percent density: pubertal staging

Associations between percent density and Tanner Stage of breast development and menarche at start of treatment were examined within the treated group. Square root percent density was less in girls treated after Tanner Stage 2 (breast) (age, BMI and number of livebirths adjusted regression coefficient –0.19 (95% CI: –1.14 to 0.76) compared to those who were treated before this stage, however, this effect was not statistically significant (p=0.69). The direction of the association between percent density and Tanner Stage at start of treatment opposed that observed with dense area. Similarly, there was no meaningful difference in mean percent density between women treated before and after menarche; age, BMI and number of livebirths adjusted regression coefficient: –0.02 (95% CI: –0.56 to 0.51) (p=0.92).

7.3.7.3 Multivariable analysis: non-dense area

Table 7.20 presents the adjusted regression coefficients for the treatment effect on non-dense area (cm^2) (log). Additional covariates (listed in **Tables 7.1** to **7.5**) were added to the regression model one at a time to observe their effect on the coefficient of the treatment variable. Only age, BMI and number of livebirths were found to significantly influence the treatment effect. The coefficients tabulated in **Table 7.20**, suggest that non-dense area is slightly but insignificantly greater in treated women compared with untreated women.

Table 7.20: Univariable and multivariable regression coefficients of the association between treatment and log non-dense area (cm^2).

Variables	Regression coefficients (SE)			
Treatment	**–0.08 (0.08)**	**–0.14 (0.08)**	**0.01 (0.06)**	**0.02 (0.06)**
Age	–	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)
BMI*	–	–	–25.3 (1.64)	–25.5 (1.66)
Livebirths†	–	–	–	0=0.00 1= – 0.07 2= – 0.01 ≥3= 0.04
P-value for Tx	0.32	0.07	0.90	0.78
95% CI	–0.23 to 0.08	–0.30 to 0.01	–0.14 to 0.10	–0.10 to 0.14

* BMI is square root inverse transformed

† Categorical variable: livebirths 0=0, 1=1, 2=2 3>=3

The adjusted coefficients were used to calculate least square means to aid in their interpretation. Mean non-dense area adjusted for age, BMI and number of livebirths was 71.7 cm^2 (95% CI: 66.2 to 77.7) in treated women, and 70.5 cm^2 (95% CI: 64.7 to 76.9) in untreated women. On average, treated women had 1.2 cm^2 greater non-dense area, adjusted for age, BMI and livebirths, compared with untreated women, though this difference was not statistically significant (p=0.78).

Postestimation diagnostics: non-dense area

Post estimation diagnostics and sensitivity analysis was performed as for dense area and percent density. No influential points were found to significantly change the results. Removing digital images from the analysis did not affect the results. While the assumptions

of linearity and normality were found to be fulfilled for the independent variables age and livebirths, this was not the case for BMI. While BMI and non-dense area are positively correlated, the relationship is curvilinear (the gradient is reduced at the larger end of the BMI scale). For BMI adjusted analyses, where the response variable is non-dense area, an inverse square root transformation of BMI was carried out to meet the assumptions of linearity. This transformation was determined using a fractional polynomial technique[461]. To illustrate this association, component plus residual plots for BMI are presented in Appendix 18.

Treatment type

Table 7.21 contains the results for the unadjusted and adjusted analysis for treatment type (DES vs no treatment, EE vs no treatment) on non-dense area (log).

Table 7.21: Unadjusted and adjusted regression coefficients of non-dense area (cm^2) (log) by treatment type: diethylstilbestrol (DES) and ethinyl estradiol (EE).

Treatment type*	Unadjusted coefficient (95% CI)	P-value	Adjusted † coefficient (95% CI)	P-value
DES	–0.002 (–0.18 to 0.18)	0.98	–0.03 (–0.18 to 0.13)	0.74
EE	–0.13 (–0.33 to 0.07)	0.22	0.09 (–0.07 to 0.24)	0.27

* Categorical variable, 1=no treatment, 2=DES, 3=EE.

† Adjusted for age, BMI, number of livebirths

It appears that ethinyl estradiol has a slightly larger positive coefficient for non-dense area than that for DES, though this is only marginal.

Treatment duration and effectiveness: non-dense area

Regression coefficients are presented in **Table 7.22** for duration of treatment and treatment effectiveness (EMH minus final height), and suggest no association between duration of treatment or treatment effectiveness with non-dense area (log).

Table 7.22: Adjusted regression coefficients of associations between non-dense area (cm^2) (log) and a) duration of treatment and b) estimated mature height (EMH) minus final height (cm).

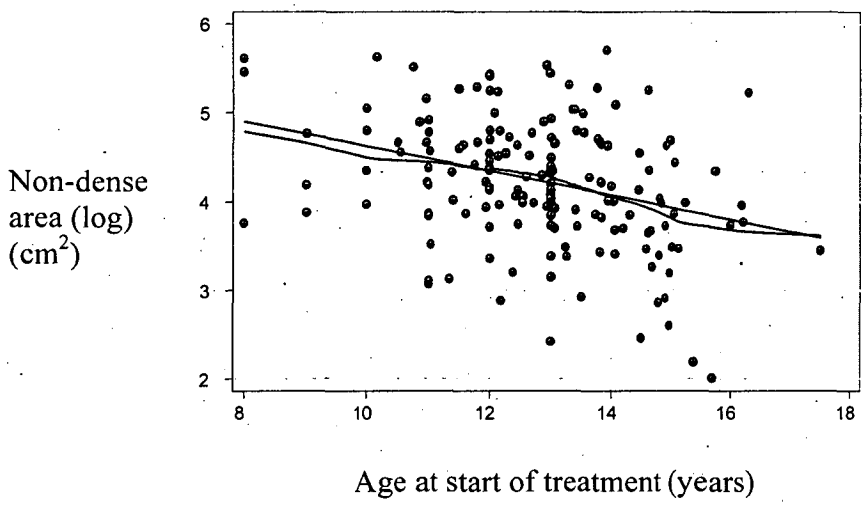
	Coefficient (95% CI)	P-value	N
Duration of treatment (years)*	–0.01 (–0.14 to 0.13)	0.91	111
EMH minus final height (cm)*	–0.002 (–0.04 to 0.04)	0.93	114

* Adjusted for age, BMI and number of livebirths

Age at start of treatment: non-dense area

Log transformed non-dense area was significantly and negatively associated with age at start of treatment (regression coefficient –0.14 (95% CI: –0.20 to –0.08) ($p<0.001$) (See **Figure 7.15**). This association was diminished when adjusted for BMI: –0.05 (95% CI: –0.10 to 0.01) ($p=0.16$).

Figure 7.15: Smoothed regression and lowess curves for non-dense area (log) (y-axis) and the age at beginning of treatment (years) in treated women.



Treatment timing on non-dense area: pubertal staging

Women who started treatment at Tanner Stage 5 have a greater non-dense area than those who began treatment at Stages 1 or 2: regression coefficient 0.31 (95% CI: –0.05 to 0.66) (p=0.09). This effect is heightened when adjusted for age at start of treatment: 0.57 (95% CI: 0.16 to 0.97) (p=0.01).Women who started treatment before menarche (n=81) have a slightly lower mean non-dense area 82.0 cm^2 (SD 51.9), compared to those who started treatment following menarche 92.6 cm^2 (SD 67.5) (n=85) but this difference is not significant (p=0.71).

7.3.7.4 Multivariable analysis: total breast area

Table 7.23 presents the adjusted regression coefficients for the treatment effect on total breast area (cm^2) (log). Additional covariates (listed in **Tables 7.1** to **7.5)** were added to the regression model one at a time to observe their effect on the coefficient of the treatment variable. Only age and BMI were found to significantly influence the treatment effect. The coefficients tabulated in **Table 7.23**, suggest that total breast area is smaller in treated women compared with untreated women but this difference does not remain after adjustment for BMI.

Table 7.23: Unadjusted and adjusted regression coefficients for treatment effect on total breast area (cm^2) (log transformed).

	Regression coefficients (SE)		
Treatment	–0.09 (0.05)	–0.12 (0.05)	–0.03 (0.04)
Age	–	0.01 (0.01)	0.01 (0.004)
BMI	–	–	0.06 (0.003)
P-value for treatment	0.07	**0.03**	0.41
95% CI	–0.19 to 0.01	–0.22 to –0.01	–0.11 to 0.05

The coefficients for treatment effect on total breast area in the age and BMI adjusted regression equation presented above were used to calculate least square means to aid in their interpretation. Mean total breast area adjusted for age and BMI was 105.6 cm^2 (95% CI: 100.1 to 111.4) in treated women, and 109.3 cm^2 (95% CI: 103.1 to 115.8) in untreated women. The least square means of total breast area adjusted for age and BMI suggest a difference of just under 4 cm^2 between treated and untreated women. This is small in breast terms, and the confidence intervals overlap suggesting a small but insignificant difference between the groups.

Postestimation diagnostics: total breast area

Post estimation diagnostics were performed and the assumptions of linearity, and normality were found to be fulfilled. The removal of influential observations as identified by Cooks D (n=9), digital images (n=21) or breast cancer cases did not significantly alter the coefficients. These diagnostics provide added confidence to the regression findings.

Treatment type

Table 7.24 contains the unadjusted and adjusted analysis for treatment type (DES vs no treatment, EE vs no treatment) on total breast area (log).

Table 7.24: Unadjusted and adjusted regression coefficients of total breast area (cm^2) (log) by treatment type: diethylstilbestrol (DES) and ethinyl estradiol (EE).

Treatment type*	Univariable coefficient (SE) (95% CI)	P-value	Multivariable † coefficient (SE) (95% CI)	P-value
DES	– 0.07 (–0.19 to 0.05)	0.24	–0.04 (–0.15 to 0.06)	0.44
EE	0.11 (–0.24 to 0.03)	0.12	–0.01 (–0.12 to 0.09)	0.85

* Univariable categorical variable, 1=no treatment, 2=DES, 3=EE.

† Adjusted for age, BMI

There appears to be no difference between the two types of treatment on total breast area.

Duration and effectiveness of treatment: total breast area

Regression coefficients are presented in **Table 7.25** for duration of treatment and treatment effectiveness (EMH minus final height) with total breast area, and suggest no association between either variable with total breast area.

Table 7.25: Adjusted regression coefficients of associations between total breast area (cm^2) (log) and a) duration of treatment and b) estimated mature height (EMH) minus final height (cm).

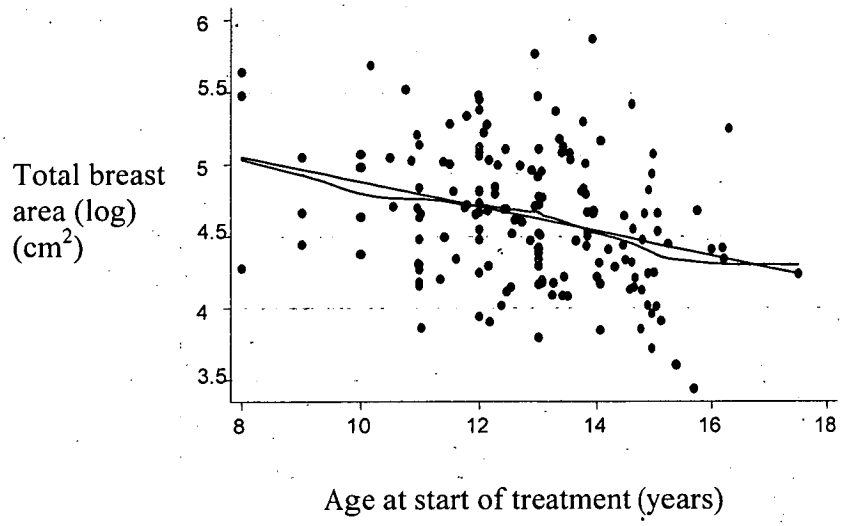
	Coefficient (SE) (95% CI)	P-value	N
Duration of treatment (years)*	–0.03 (0.04) (–0.12 to 0.05)	0.46	111
EMH minus final height (cm)*	0.003 (0.01) (–0.02 to 0.03)	0.81	114

*Adjusted for age and BMI

Age at start of treatment: total breast area

Log transformed total breast area was significantly and negatively associated with age at start of treatment (regression coefficient –0.09 (95% CI: –0.13 to –0.05) ($p<0.001$) (**Figure 7.16**). This effect was reduced when adjusted for BMI: coefficient –0.03 (95% CI: –0.06 to 0.01) ($p=0.16$).

Figure 7.16: Smoothed regression and lowess curves for total breast area (log) (y-axis) and the age at beginning of treatment (years) (x-axis) in treated women.



Treatment timing on total breast area: pubertal staging

Women who started treatment at Tanner Stage 5 had a greater total breast area than those who began treatment at Stages 1 or 2 when adjusted for age and BMI regression coefficient 0.12 (95% CI: 0.004 to 0.47) (p=0.05). This effect could be due to an earlier age at start of treatment. When adjusting for this, the size of the effect was increased (regression coefficient: 0.42 (95% CI: 0.15 to 0.69) (p=0.003). This suggests that those women who started treatment later in development had greater total breast area than those who started early in breast development.

Total breast area is similar whether women started treatment before or after menarche age and BMI adjusted regression coefficient: 0.01 (95% CI:–0.10 to 0.13); age, BMI and age at start of treatment adjusted: –0.004 (95% CI: –0.12 to 0.11)

7.3.7.5 Summary of multivariable analyses for all outcome measures

For comparative purposes, a summary table of the final multivariable models for treatment effect on dense area, percent density, non-dense area and total breast area is provided below (**Table 7.26**). As outlined above, age and BMI were retained for dense area and total breast area, and age, BMI and number of livebirths for percent mammographic density and non-dense area. Use of the alternative definition of menopausal status (see methods Section 7.2.6.1) resulted in 23 (13.8%) treated women and eight (5.6%) untreated being classified as postmenopausal. This alternative definition did not change the results for dense area, percent density, non-dense area or total breast area.

Table 7.26: Multiple linear regression of the association between treatment and each of the mammographic measures adjusted for different sets of covariates*.

Mammographic measure and covariates	Regression coefficient	95% CI	P-value
Dense area †			
Age, BMI	–0.45	–0.86 to –0.04	0.032
PMD †			
Age, BMI, live births	–0.28	–0.67 to 0.11	0.16
Total breast area ‡			
Age, BMI	–0.03	–0.11 to 0.05	0.41
Non-dense area ‡			
Age, BMI, live births	0.02	–0.10 to 0.14	0.78

* Age (years), BMI (kg/m^2), HRT (current), menopause (postmenopausal if last period ≥52 weeks, and if HRT started before last period and ≥55 years)

† Square root transformed; ‡Log transformed

A summary table of adjusted regression coefficients for the effect of high-dose estrogen treatment on square root dense area, square root percent density, log total breast area and log non-dense area is presented below (**Table 7.27**).

Table 7.27: Adjusted least square means of total breast area (cm^2), non-dense area (cm^2), percent density (%) and dense area (cm^2) for treated and untreated women.

	Treated mean (95% CI)	Untreated mean (95% CI)
Total breast area (cm^2)*	105.6 (100.1 to 111.4)	109.3 (103.1 to 115.8)
Non-dense area (cm^2) †	71.7 (66.2 to 77.7)	70.5 (64.7 to 76.9)
Percent mammographic density (%) †	24.8 (22.4 to 27.4)	27.7 (24.8 to 30.7)
Dense area (cm^2)*	24.5 (21.8 to 27.2)	29.1 (26.0 to 32.4)

* Adjusted for age, BMI.

† Adjusted for age, BMI and number of livebirths.

7.4 Discussion

This is the first study to examine the long-term effects of high-dose estrogen exposure in adolescence on mammographic density. This study found that treated women had a significantly lower mean dense area than women who were also assessed for tall stature but untreated. Treated women had less mean total breast area and percent density, and slightly greater mean adjusted non-dense area compared with untreated women but these differences were not statistically significant.

Hormone replacement therapy is known to increase mammographic density[343] yet this study found women treated with high-dose estrogens (plus a cyclic progestagen) in adolescence had reduced mammographic density as an adult, compared with untreated women. However, these results are plausible. Puberty in girls treated with high-dose estrogen is accelerated. Treatment induces menarche earlier, and closes the epiphyses of the long bones, as typically occurs at the end of puberty. Given that dense area for age tracks through adulthood[462], the lower mean adult dense area observed for treated women suggests less net growth in dense area during puberty, consistent with accelerated maturation. This is also consistent with the many cross-sectional observations that earlier age at menarche is associated with reduced adult mammographic density[407-409, 463, 464].

There are a number of biological mechanisms that might explain the observed effect on mammographic density in this study. High-dose estrogens may have a direct inhibitory effect on the developing breast, in particular the epithelial and stromal tissue that makes up the dense part of the breast. This effect may be mediated by reduced levels of insulin-like growth factor (IGF-I) observed in treated girls during treatment[10-13, 91]. While, IGF-I has been positively associated with mammographic density[465-467], no studies have examined the association between IGF-I levels during adolescence and mammographic density as an adult. Kleinberg and co-authors have highlighted the importance of IGF-I in ductal morphogenesis during pubertal mammary development in IGF-I insufficient animals[468].

This relationship between dose of estrogen and IGF-I response is dependent on age and dose. Rooman and colleagues[10] reviewed a number of studies on estrogen and subsequent

effects on IGF-I levels and observed that in children and adolescents, low doses of estrogen (up to 0.030 mg) increase serum IGF-I while higher doses (higher than 0.100 mg) decrease IGF-I levels. In adults, this biphasic effect is not as evident. Both low-dose estrogens (e.g. oral contraceptives or HRT) and high-dose estrogens as observed in human volunteers, in the treatment of acromegaly or male to female trans-sexuals, decrease IGF-I levels[10].

Unfortunately, plasma IGF-I levels were not collected from the women in this study at time of treatment, therefore there is no capacity to examine the possibility that the reduction in dense area might be mediated by a reduction in IGF-I.

Another potential mechanism for the effect of high-dose estrogen in adolescence on mammographic density as an adult is the suppression of ovarian function, suggested by the published reports of a reduction FSH and LH levels in treated girls (See Chapter 2, Section 2.4.2.3). GnRH agonists have been shown to reduce mammographic density[469] (See Section 6.4.1.2 of Chapter 6). One possibility is that the reduction in ovarian function that occurs with high-dose estrogen treatment in adolescence, contributes to the overall reduction in dense area observed in treated women in this study.

Some case-series reports of the outcomes of treatment for tall stature have noted some girls' concerns about a lack of breast development[41,23]. This may explain some or all of the reduction in dense area observed in the treated women of this cohort. As percent density was reduced, it appears that at least some of the reduction in dense area was independent of total breast area. Adjusting for total breast area, did not reduce the treatment estimate on dense area.

Dense area and percent mammographic density were negatively associated with duration of treatment, though these associations were not statistically significant, possibly due to the smaller sample size after restricting the analysis to individuals with medical record data for this parameter.

In this study, a positive association was observed between age at start of treatment and dense area. It is possible that girls treated at an earlier age matured earlier (the majority

of girls were treated on or after Tanner Stage 3 of the breast). The positive association between dense area and age at start of treatment was removed once adjusted for BMI, supporting the early maturity effect. That is, if girls started treatment later, they are likely to have matured later also. They had more time for mammographic density development consistent with the established positive association between age at menarche and mammographic density[302, 328, 359, 407, 409, 444] described above. Adjustment with BMI removed the effect observed between dense area and age at start of treatment possibly because adult BMI is associated with timing of pubertal maturity. Adult BMI is highly correlated with BMI during adolescence[470, 471], and it is reported that high BMI in adolescence is correlated with early age at menarche[470, 471]. As well, adult BMI has been inversely associated with age at menarche[472], an indicator of early sexual maturity. Correspondingly, non-dense area and total breast area were both significantly and negatively associated with age at start of treatment. This association also diminished when adjusted for BMI.

A positive association was observed between total breast area and Tanner Stage of breast development at start of treatment. Women who started treatment at Tanner Stage 5 had a greater total breast area than those who began treatment at stages 1 or 2 when adjusted for age and BMI. This effect could be due to an earlier age at start of treatment. When adjusting for this, the size of the effect was strengthened. A similar finding was observed for non-dense area. These findings suggest that those women who started treatment later in development had greater total breast area (and non-dense area) than those who started at the early stage of breast development. It would be expected then, that treated women would have a lower mean total breast area than untreated women. A lower mean total breast area was observed in untreated women compared to treated women (adjusted for age), but this effect was no longer statistically significant after further adjustment for BMI. These findings are consistent with those described in Chapter 5. This chapter reported no difference in the proportion of treated and untreated women whose breasts did not increase in size during pregnancy (a symptom of breast hypoplasia). Also, the findings reported here in relation to total breast area, do not support the anecdotal reports that treatment caused flat-chestedness in girls, at least in the longer term.

Findings from the first follow-up of Tall Girls[77], have shown large variability in the effectiveness of treatment in reducing adult height, with a mean difference between the

estimated mature height (EMH) and final height in the treated group of –2.5 cm (95% CI: –3.2 to 1.8). Clinical effectiveness of estrogen treatment on final height reduction (EMH–final height) did not appear to be associated with any of the mammographic parameters, suggesting that any treatment induced change in the mammographic parameters occurred independently of the effect of treatment on epiphyseal closure and hence height reduction.

While dosage of estrogen was standard across the cohort, the girls' plasma levels might have varied. Brody et al. (1989)[473], observed extreme inter and intra-variability in plasma levels following doses of 35 μg ethinyl estradiol (compared to the 150 μg typically given to treated tall girls in this cohort). Maximum plasma levels ranged between 55–311 pg/ml in different individuals (n=24). Intra-individual variability when provided in three consecutive monthly doses at the same time of cycle, had a coefficient of variation of 41% or range of –79% to +134% around the mean of the per individual[473]. According to Goldzieher[474], the "...range of inter and intra-individual variability [in plasma levels] have important clinical implications. This accounts for the well-established observation that the same dose may produce overdosage effects (e.g. nausea) in one person and underdosage effects (e.g. breakthrough bleeding) in another. It also points out that efforts at fine-tuning dosage to minimise adverse effects of either variety are not going to succeed except "on the average" Goldzieher argued that this variability is not due to differences in the absorption and excretion components of pharmacokinetics but rather, through differences in metabolism.

This might explain why some girls developed side effects with treatment while others did not. Seventy-five treated women in this cohort experienced breast related side effects during treatment as an adolescent girl (e.g. galactorrhoea, dry and cracked nipples and breast pain). Of interest is the observation made by two studies that breast pain with HRT use is associated with an increase in mammographic density. In a prospective study of HRT users, McNicolas et al.[243] observed that seven of nine women (78%) who had experienced an increase in mammographic density after commencing HRT, had also experienced moderate or severe breast pain. In contrast, only five of 24 (21%) of those who did not experience an increase in mammographic density experienced moderate or mild breast pain. No women belonging to a control group of non-HRT users (n=31) reported breast pain during the same follow-up period.

Crandall et al. 2006[241], observed a similar association in a subset of the PEPI RCT cohort. Women who experienced the onset of breast discomfort within a 12 month period following HRT commencement, had a 3.9% increase (age and multivariable adjusted) in mean percent mammographic density following HRT use, compared with an 0.6% increase in women who did not experience breast discomfort ($p<0.001$).

It is possible that an increase in breast pain during treatment coincided with an increase in mammographic density. In this study, the 12 treated women who experienced breast pain as a side effect had a higher dense area (sqrt) compared to women who did not experience breast pain during treatment but this difference was not significant ($p=0.26$) and the numbers were too small to be confident with this finding. Including women in the sample who experienced any breast related side effect during treatment would increase the sample size to 75. It is possible that this group had higher plasma levels of estrogen compared with treated girls who did not experience breast related side effects. There was no difference in dense area (sqrt) in treated women who experienced any breast related side effect during treatment (e.g. galactorrhoea, breast pain or dry and cracked nipples) compared with treated women who did not.

The mean levels of each of the mammographic density parameters in this study are similar to those reported by Boyd et al. (2002)[392] for women of a similar mean age (44.8 years). Boyd et al. reported mean values for each of the mammographic measures using a similar computer assisted thresholding technique to measure density. Mean percent density in their study was 28.5% and dense area 30.2 cm^2, non-dense 101.43 cm^2, and total breast area 131.63 cm^2. In comparison the mean age of women in this PhD study was 48.4 years. Mean percent density was 29.5% for treated and 30.8% for untreated, dense area was 27.6 cm^2 for treated and 32.8 cm^2 for untreated, while non-dense area was 87.0 cm^2 and 91.1 cm^2 total breast area was 114 cm^2 for treated and 123 cm^2 for untreated women.

Dense area and total breast area in this study were adjusted for age and BMI, while percent mammographic density and non-dense area were adjusted for age, BMI and number of livebirths as they were found to warrant inclusion in the regression equations for treatment effect on these parameters. Age has previously been reported to be a strong predictor of dense area[407, 475, 476], percent density[305, 306, 325, 327, 328, 359, 407, 409, 413, 440-443], and non-dense area[407, 476].

BMI has been strongly associated with percent density[305, 325, 328, 359, 360, 407, 409, 413, 442, 444], non-dense area[407, 408, 476, 477] and total breast area[477] and to a greater[475] or lesser[408, 477] extent, dense area. Number of livebirths has also been previously associated with percent density[305, 306, 328, 359, 360, 407, 409, 413, 440-444] and non-dense area[407, 408, 476]. Age at menarche was not found to contribute to the estimates for any of the mammographic measures. Age at menarche in treated girls is not an accurate measure for treated girls who started treatment before natural menarche. In these girls, treatment induced menstruation.

HRT use, both current and past use, was greater in treated women. It is also associated with mammographic density as reviewed extensively earlier in Chapter 6 (Section 6.4.1.1) however, despite HRT use being a potential confounder, it did not have any effect when included in the regression for treatment on either of the mammographic measures. This is not all that unexpected. It has not always been found to be an important predictor of mammographic density. According to Ursin and Pike[478], although mammographic density can be increased by hormone therapy exposure, the effects of these exposures are only ~5% (See **Table 6.3** in Chapter 6), "the contribution of hormonal exposure in determining the variation in the distribution of mammographic density at any point in time is therefore quite small".

To interpret the findings it helps to consider the potential mechanism for any covariates included in the regression equation. A decision will need to be made as to the effect of adjustment of variables that may share risk factors with the outcome of interest as well as be affected by the exposure variable. As stated by Wilcox (2006)[479] , investigators should "Never adjust for covariates just because they are handy, Epidemiologists cannot depend on adjustments (or stratifications of any sort) to bring results closer to the truth" p 1,123. Before adjusting for a variable, it is important to consider its role in the causal pathway.

Age and BMI were included in the final regression models for treatment effect on dense area, percent density, non-dense area and total breast area. Number of livebirths was included in the regression model for percent density and non-dense area. Age possibly acted as a confounder because it was negatively associated with dense area, percent density and non-dense area as demonstrated by the univariable analysis and reports elsewhere, and was

associated with treatment status (treated women were on average older than the untreated women). This difference in mean age between treated and untreated women was possibly because fewer tall girls accepted treatment in later years.

BMI was associated with each of the mammographic outcome measures and differed between the groups. It was possibly associated with the offer of treatment for tall stature. Untreated girls might have reached maturity earlier than treated girls, leaving them with little growth potential to warrant treatment at time of assessment. As stated above, early maturity (puberty) is associated with higher BMI in adolescence and adolescent BMI has been shown be highly correlated to adult BMI.

Number of livebirths might have acted as an effect mediator. It have been previously reported to be influenced by treatment[25] and has been found to have an inverse relationship with mammographic density. Adjusting for livebirths did not influence the association observed between treatment and dense area. Similarly, benign breast disease and endometriosis might have acted as effect mediators. However, adjusting for these variables strengthened the association between treatment and mammographic dense area.

There are a number of limitations of the study that need to be considered. Firstly, there is the possibility that selective participation may have introduced bias. Women are generally not aware of the degree to which their breasts are dense, and are therefore unlikely to select themselves on the basis of this knowledge. However, women with breast problems may have been more receptive to the invitation to participate in the study. Participants were no more likely to have had a breast biopsy at time of the original study than non-participants. A history of biopsies for benign breast disease has been associated with an increase in percent mammographic density[390, 480]. While more treated than untreated women in this study reported a previous diagnosis of benign breast disease, any selection bias in this regard would have led to an underestimation of the effect of treatment on mammographic density. Adjusting for a history of benign breast disease, amplified rather than attenuated the estimate for treatment effect on dense area.

The findings could be prone to selection bias due to differential non-response in the original follow-up of the Tall Girls Study cohort since 28% of treated and 44% of untreated women did not agree to participate. However, we are interested in associations not prevalence estimates, so non-response would only be a problem if it were systematically associated with both the predictor and outcome variables of interest. There is no obvious plausible reason why women in the treated and untreated groups in the earlier cohort, from which this study sample was derived, would vary in their willingness to participate in ways that were differentially related to mammographic density. Earlier findings on impaired fertility were largely unaffected by non-response and the inclusion or exclusion of self-referred women[25]. A sensitivity analysis was performed by removing women who self-referred to this mammographic study from the dataset. This made no difference to the results.

Women are not generally aware of the degree to which their breasts are dense, and are therefore unlikely to select themselves on the basis of this knowledge. However, it is possible that women lost to the study, or not participating to the study had breast cancer, and therefore had higher levels of mammographic density. Removing all participants who had a history of breast cancer from the analysis (more treated than untreated) did not affect the results.

The observed effect of adolescent exposure to high-dose estrogens on dense area cannot be generalised to all exogenous estrogen exposures that might occur during adolescence (e.g. diet or the environment). Estrogen has a biphasic effect on some tissues[7] exerting a different action at low concentrations than at high concentrations. For example, at low plasma concentrations endogenous estrogen is believed to stimulate the growth spurt at the start of puberty, while at higher concentrations, at the end of puberty, endogenous estrogen plays a role in the cessation of growth. It may follow therefore, that lower exogenous estrogen exposures during adolescence may have a different effect on mammographic density than that observed with the high estrogen exposures in this study. Receptor mediated responses do not always follow linear one-way dose-responses that are often assumed with hormonal exposures. Welshons and colleagues[481] have argued that studies should not extrapolate lower dose effects linearly from high-doses or assume the dose-response relationships are monotonic. Receptor-mediated responses do saturate at some point, and can increase then decrease as the dose increases[481].

The oral contraceptive pill contains ethinyl estradiol in much smaller doses than those used in the treatment of tall stature in our study. Overall evidence suggests that oral contraceptive use at a younger age (before 20 years) is associated with a greater risk of breast cancer than use at a later age[178-180] . Whether early use of oral contraceptives also equates to a corresponding increase in mammographic density is unknown. One study found no association between oral contraceptive use as a young adult and percent mammographic density later in life[360].

The exploration of the association between estrogen treatment in adolescence and mammographic density as an adult involved multiple comparisons. The association between mammographic density and different forms of treatment (e.g. DES, EE, and both combined) were explored as was different measures of mammographic density (percent density and dense area) with treatment. Since one in twenty comparisons is expected to be statistically significant at the 5% level by chance alone (Kirkwood and Sterne, 2002), it is expected that the greater the number of comparisons, the greater the number of potential statistically significant results by chance alone. Some researchers attempt to address this problem by adjusting for multiple comparisons. This approach was not adopted in this study. Rothman argues that adjustments for multiple comparisons should not be performed. He purports that each association should be considered 'on its own for the information it conveys'. In addition, adjustment for multiple comparisons, while attempting to reduce the chance of type I error, can increase the frequency of incorrect rejections of the null hypothesis (type II error) (Rothman, 1990). Consistent with Rothman's view, this thesis reports the results for all multiple comparisons, thus allowing the reader to quantify the number of tests and make their own adjustments for multiple testing". p258 Second last paragraph of discussion (Section 7.4).

The difference in dense area between treated and untreated women might be due to differences in pre-treatment characteristics. Childhood growth parameters (e.g. birthweight, birthlength, and growth-velocity) have been associated with mammographic density and

differences in these factors might account for the differences observed in dense area between treated and untreated women. If these parameters were independently associated with treatment status and mammographic density, this might explain the observed association (dotted line) between treatment and mammographic density. If this was indeed the mechanism then further adjustment for these parameters would reduce the association between treatment status and mammographic density. The next chapter explores a number of pre-treatment growth parameters and their influence on the treatment effect on mammographic density.

7.5 Conclusion

This study found treated women to have a significantly lower mean dense area than women who were also assessed for tall stature but untreated. Treated women had less mean total breast area and percent density, and slightly greater mean adjusted non-dense area compared with untreated women but these differences were not statistically significant. These findings are reassuring for treated women, however, the difference in dense area between treated and untreated women might be due to differences in pre-treatment characteristics. This possibility is explored further in Chapter 8.

Box 7.1: Summary of key chapter findings

KEY FINDINGS: CHAPTER 7

- Treated women had a significantly lower mean mammographic dense area than women who were also assessed for tall stature but untreated.
- Treated women had less mean adjusted total breast area and percent density, and slightly greater mean adjusted non-dense area compared with untreated women but these differences were not statistically significant.
- Dense area was not associated with duration of treatment.
- A positive association was observed between age at start of treatment and dense area. Since early maturity corresponds with earlier age at start of treatment, this suggests that dense area is higher in women who matured later, consistent with the observation elsewhere that mammographic density is positively associated with age at menarche.
- A positive association was observed between total breast area and Tanner Stage of breast development at start of treatment, after adjustment for age and BMI. This effect remained after adjustment for age at start of treatment. A similar finding was observed for non-dense area. These findings suggest that those women who started treatment later in development had greater total breast area (and non-dense area) than those who started at the early stage of breast development.

8: CHILDHOOD AND ADOLESCENT GROWTH PARAMETERS, AND MAMMOGRAPHIC DENSITY IN TREATED AND UNTREATED WOMEN

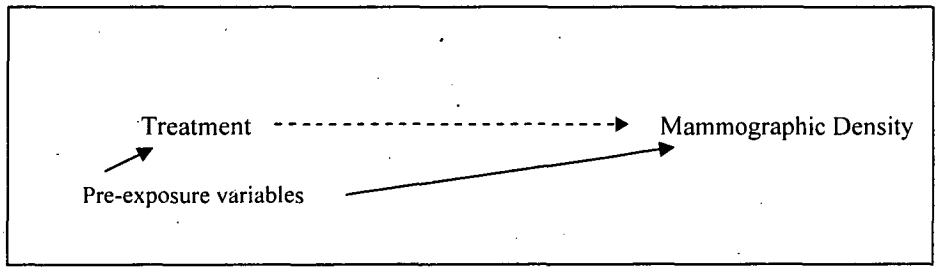
8.0 Introduction

Chapter 7 has shown that women treated for tall stature in adolescence have less dense area than untreated women. Treated women might differ from untreated women in some pre-treatment growth parameters associated with mammographic density (e.g. birthweight, birth-length, growth velocity, or childhood height). From previous reports it is known that untreated girls generally have less growth potential at time of assessment than treated girls, reflected by a greater level of pubertal maturity and more advanced bone age when compared to their chronological age[77]. If any of these variables are associated with mammographic density and they differ between treated and untreated women, they might explain the differences in mammographic density observed between treated and untreated women.

Chapter 6 summarised studies that examined associations between a number of childhood anthropometric variables and mammographic density. A number of these were found to be positively associated with mammographic density as an adult (birthweight[431], childhood height[419], weight[419, 426] and height velocity[406]). In contrast, the studies by McCormack et al. (2003)[406], Sellers et al. (2007)[419] and Samini et al. (2008)[426] each observed an inverse association between childhood BMI or weight and mammographic density as adult. As mentioned in Chapter 6, a more detailed review of these and other associations is in Appendix 8.

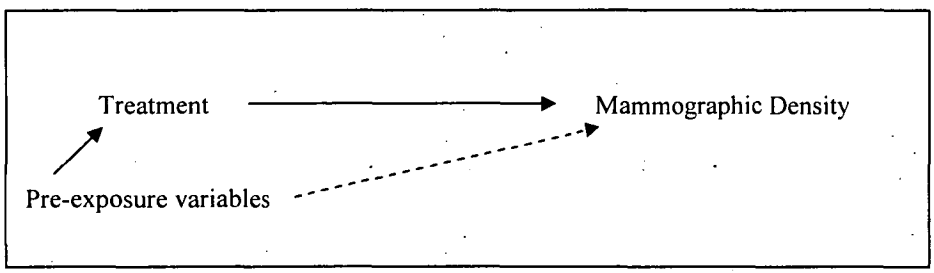
Pre-treatment growth related variables (e.g. bone age, birthweight) could be potential confounders if they are associated with the exposure of interest (treatment) and independently associated with the outcome of interest (mammographic density)[482]. In the scenario illustrated in **Figure 8.1** below, treatment and mammographic density might be statistically associated because they have a common cause (pre-exposure variables), even if the causal pathway signified by a dotted arrow does not exist. Any analysis of the association between treatment and mammographic density should adjust for these factors if possible.

Figure 8.1: Directed acyclic graph illustrating confounding of the association between treatment and mammographic density by pre-exposure variables.



Caution is needed when interpreting the effect of these added variables on the association between treatment and mammographic density. If, for example, pre-exposure factors are only linked along the causal pathway to treatment (bold arrows; **Figure 8.2**) but not the outcome of interest (dotted arrow, **Figure 8.2**) then adjusting for the pre-exposure variable might reduce or remove the visible association between treatment and mammographic density. If this was to occur, it might appear that the pre-exposure variable was confounding the observed association when in fact it was not. For it to be considered a confounder, it is important that the pre-exposure variable has an independent association with mammographic density if its addition to the regression alters the association in any way.

Figure 8.2: Directed acyclic graph illustrating a non-confounding scenario of the association between treatment and mammographic density.



On the other hand, post-treatment variables such as weight and BMI change and age when maximum height was attained following treatment might explain the association

between treatment and mammographic density observed in the previous chapter. That is, treatment might not act directly on mammographic density; instead it might act indirectly through any changes on these growth parameters. If this is so, these variables would be mediators.

The Tall Girls Study collected a number of growth related variables from the medical records of study participants. This rich data-set provided the opportunity to explore the independent effects of a number of childhood growth variables on mammographic density. In follow-up 2, additional variables were also extracted from the medical records of the participants who had previously provided their consent to extract information from the records (e.g. birthweight and birth-length).

This chapter reports on the association between a number of childhood anthropometric variables and mammographic density at follow-up and the effect of some of these variables on the observed association between treatment and mammographic density.

8.1 Study Aim

There are two main aims to this chapter:

1. To examine the degree to which a number of pre-treatment growth parameters such as birthweight, birthlength, childhood height, BMI, and bone age might influence the association observed between treatment and mammographic density.

2. To examine the association between a number of adolescent anthropometric measures that might have been influenced by treatment (e.g. BMI change, age at which maximum height was attained) on each of the mammographic measures for treated women only.

8.2 Method

Childhood anthropometric data were collected from the medical records of participants who provided their written consent. Bone age and height, weight and BMI at first assessment were collected from the records at first follow-up, and birthweight, birth-length, and the variables derived from multiple height and weight data were collected from the records at second follow-up. Each variable is described in more detail in the following sections.

8.2.1 Birthweight and birth-length data

Birthweight and birth-length data were often, but not always, recorded in the medical records by the treating endocrinologist. Birthweight data were available for 184 participants (59.5%) (72 treated and 112 untreated). Birth-length data were available for 106 participants (34.3%) (48 treated and 68 untreated).

The specificity of the data suggests that birthweight and birth-length were extracted from birth records of the child, however, it is likely that some parents did not present their child's birth records to the endocrinologist, and instead relied on memory to report birthweight and birth-length. The endocrinologist's records did not specify the source of the data.

Data were recorded in stones and pounds or kilograms (kg). Stones and pounds were converted to kilograms. Birth-length data were recorded in feet and inches or centimetres (cm). Feet and inches were converted to centimeters.

8.2.2 Bone age data

Bone age was derived from wrist x-rays as described in detail in Chapter 2 (Section 2.3.1). If bone age was greater than a child's chronological age, then they were considered to have less

growth potential and to have greater skeletal maturity compared with a child of the same age, whose bone age is similar or less than their chronological age.

Bone age was routinely recorded in the medical records by the treating endocrinologist. In this cohort, bone age measurements were available for 237 (76.7%) participants; treated n=109 (65.3%), untreated n=128 (90.1%). Age at which the wrist x-ray was taken (chronological age) was derived from the date of x-ray and date of birth.

8.2.3 Height, weight and BMI at first assessment

When the girl first presented to the endocrinologist, height and weight were recorded in the medical records. Height was recorded in feet and inches or centimetres. Weight was recorded in stone and inches or kilograms. Conversions were made as for birthweight and birth-length. BMI (kg/m^2) at first assessment was derived from the weight and height data (height in centimetres was converted to metres).

Height at first assessment was available for 239 participants (77.3%) or 111 treated and 128 untreated women while weight (and therefore BMI) at first assessment was available for 226 participants (73.1%); 104 treated, 122 untreated. Age at first assessment was derived from the date of visit and date of birth.

8.2.4 Weight and BMI change one year following treatment

Change in weight and BMI for each treated individual was calculated by subtracting the weight and BMI at commencement of treatment from the weight and BMI one year following commencement of treatment. Sufficient pre-treatment BMI data to calculate BMI velocity was not recorded in the medical records for treated and untreated women, but BMI changes following treatment was available for treated women.

8.2.5 Age at which maximum height was attained

Maximum height was judged to be the woman's self reported current height as self reported in the CATI at second follow-up (see Methods section 7.2.6.1, Chapter 7). For many women, height measurements during adolescence stopped before maximum height was attained (61 of 90 records available). For these women, age at maximum height was judged to be greater than the age when measurements stopped, as verified by the final self-reported height. Age at maximum height was measured directly for the rest (n=29).

A binary variable was prepared where '1' corresponds to maximum attained height if <15 years (n=12), and '2' if age at maximum attained height is ≥15 years (n=78).

8.2.6 Growth after 15 years

This measure was available for 82 treated individuals who had height measurements recorded in their medical records at or close to 15 years. Height was measured as close as possible to 15 years (median=15.1, 5^{th} & 95^{th} percentiles: 14.8, 15.3). This medical record measure of height was subtracted from the participant's self-reported final height (current height).

8.2.7 Statistical analysis

Summary descriptive data on a number of childhood anthropometric characteristics of participants were undertaken beginning with the raw means or proportions with significance tests for differences between treated and untreated women. This was then followed by an analysis of the univariable association between each of the anthropometric variables and the mammographic density measures: dense area, percent density, total breast area and non-dense area. For those variables that were available for treated and untreated participants (e.g. birthweight, birth-length, bone age; and height, weight and BMI at first assessment), the degree to which they influenced the association between treatment with high-dose estrogens and each of the mammographic density measures was examined. Some childhood

anthropometric variables were available for treated women only (height, weight and BMI change after treatment, height change after 15 years, age at maximum height attainment), and were examined within this group only.

Significance tests of differences in characteristics between treated and untreated women were performed using the chi-square test for categorical data and t-test for continuous data. Univariable and multivariable associations between the dependent mammographic measures and the independent variables were performed using linear regression. Stata 9 was used for all analyses.

8.2.8 Ethics

Ethics approval was sought from (the Tasmanian Health and Medical Human Research Ethics Committee, H0008334) for this study to access data previously collected at first follow-up and again to extract additional information from the medical records (birthweight and birth-length). See Appendix 19 for the ethics approvals.

8.3 Results

8.3.1 Characteristics of study participants

Summary descriptive data of childhood anthropometric characteristics for treated and untreated women are presented in this section, beginning with the raw means or proportions and significance tests for differences between treated and untreated women.

8.3.1.1 Pre-treatment anthropometric characteristics of study participants

A summary of childhood anthropometric characteristics of participants, by treatment status, is presented in **Table 8.1.** Treated women were similar in mean birthweight, birth-length, weight and BMI and age at first assessment, and age at bone measurement compared with untreated women. Treated women, on the other hand, were taller at first assessment after adjusting for age at measurement ($p<0.001$). Skeletal maturity also differed between the groups. The bone age of untreated girls tended to be more advanced than their chronological age (bone age minus chronological age = 0.33 years (SD 0.85)) when measured at a mean chronological age of 12.1 years, whereas treated women tended to have less advanced bone age (bone age minus chronological age= –0.01 years (SD 0.85)), when measured at a mean chronological age of 12.3 years. This suggests earlier bone maturity, and hence less growth potential at a given age, in untreated girls. This is consistent with the decision not to provide treatment in these girls.

Table 8.1: Anthropometric characteristics of treated and untreated participants.

Characteristic	Treated N	Treated Mean (range)	Untreated N	Untreated Mean (range)	P-value
Birthweight (kg)	72	3.6 (2.6 to 5.4)	112	3.5 (2.2 to 5.0)	0.132
Birth-length (cm)	48	53.2 (48.3 to 57.2)	58	52.9 (48.3 to 61)	0.525
Bone age-chronological age (years)	109	–0.01 (–2.4 to 2.1)	128	0.33 (–1.6 to 3.1)	**0.002**
Age bone age measurement (years)	109	12.3 (7.0 to 16.0)	128	12.1 (4.9 to 16.9)	0.561
Age at first assessment (years)	111	12.3 (7.0 to 16.0)	130	12.1 (5.0 to 16.9)	0.470
Height at first assessment (years)	111	166.8 (125.1 to 183.2)	128	162.1 (120.2 to 184.2)	**<0.001**
Weight at first assessment (kg)	108	51.1 (26.5 to 79.5)	122	49.3 (23.1 to 78.0)	0.352
BMI at first assessment (kg/m^2)	108	18.2 (13.2 to 25.9)	122	18.5 (13.5 to 25.50)	0.168

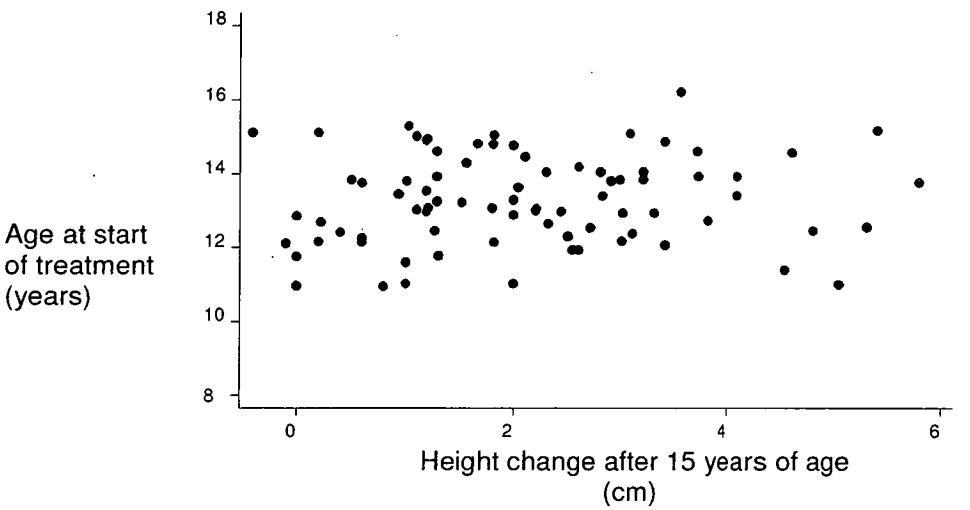
8.3.1.2 Childhood anthropometric characteristics of treated women

A number of childhood anthropometric characteristics are summarised in **Table 8.2** for treated women only. These data were not available for untreated women. Weight changes in the first year following treatment were considerable for some girls (mean 7.1 kg). Most of the girls reached their maximum height after age 15 (87% compared with 13.3%). The average height change after age 15 years was 2.1 cm. This estimation includes girls who were treated before and after 15 years. **Figure 8.3** depicts a scatter plot of the association between height change after 15 years and age at start of treatment. There appears to be no relationship between the two variables.

Table 8.2: Childhood anthropometric characteristics of treated women.

Characteristic	Treated Women
Weight change first year of treatment (kg)	
Mean (range)	7.1 (–1.6 to 17.8)
BMI change first year of treatment (kg/m^2)	
Mean (range)	1.83 (–0.73 to 5.2)
Age maximum height reached (n) (%)	
<15 years	12 (13.3)
≥15 years	78 (86.7)
Height change after 15 years (cm)	
Mean (range)	2.1(–0.4 to 5.8)

Figure 8.3: Scatter plot of age at start of treatment and height change after age 15 years.



8.3.2 Associations between childhood anthropometric variables and the mammographic density measures

A univariable analysis of the association between each of the parameters described in **Tables 8.1 and 8.2** above and each of the mammographic density measures: dense area, percent density, total breast area and non-dense area was undertaken and a summary of the findings presented in **Table 8.3.** The associations for weight and BMI change following first year of treatment, age at maximum attained height and height change after 15 years are estimated for treated women only. This data were not available for untreated women.

Table 8.3: Univariable analysis of the association between pre-treatment anthropometric measures and the outcome variables dense area (cm^2) (sqrt), percent density (%), total breast area (cm^2) (log), and non-dense area (cm^2) (log).

Covariate	Dense area		Percent density		Non-Dense Area		Total Breast Area	
	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Birthweight (kg)	0.44 (–0.10 to 0.98)	0.11	0.26 (–0.30 to 0.82)	0.36	0.02 (–0.17 to 0.21)	0.86	0.04 (–0.09 to 0.17)	0.52
Birth-length (cm)	–0.05 (–0.20 to 0.11)	0.56	–0.01 (–0.17 to 0.14)	0.89	–0.02 (–0.07 to 0.03)	0.56	–0.02 (–0.05 to 0.02)	0.42
Bone age - chronological age (years)*	–0.45 (–0.75 to –0.14)	**0.005**	–0.76 (–1.07 to –0.44)	**<0.001**	0.27 (0.16 to 0.38)	**<0.001**	0.16 (0.08 to 0.23)	**<0.001**
Height at first assessment (cm)*	–0.05 (–0.09 to –0.01)	**0.014**	–0.08 (–0.12 to –0.03)	**<0.001**	0.02 (0.01 to 0.04)	**0.002**	0.01 (0.002 to 0.02)	**0.013**
Weight at first assessment (kg)*	–0.03 (–0.06 to 0.001)	0.059	–0.08 (–0.11 to 0.05)	**<0.001**	0.03 (0.02 to 0.04)	**<0.001**	0.02 (0.02 to 0.03)	**<0.001**

Covariate	Dense area		Percent density		Non-Dense Area		Total Breast Area	
	Regression coefficient (95% CI)	**P-value**	**Regression coefficient (95% CI)**	**P-value**	**Regression coefficient (95% CI)**	**P-value**	**Regression coefficient (95% CI)**	**P-value**
BMI at first assessment (kg/m^2)*	–0.07 (–0.18 to 0.03)	0.16	–0.24 (–0.34 to –0.13)	**<0.001**	0.11 (0.07 to 0.14)	**<0.001**	0.07 (0.05 to 0.10)	**<0.001**
Weight change first year of treatment (kg) †	0.04 (–0.07 to 0.14)	0.48	0.07 (–0.05 to 0.18)	0.25	–0.02 (–0.06 to 0.01)	0.19	–0.01 (–0.04 to 0.01)	0.38
BMI change first year of treatment (kg/m^2) †	0.11 (–0.22 to 0.44)	0.51	0.19 (–0.17 to 0.55)	0.29	–0.07 (–0.19 to 0.05)	0.23	–0.03 (–0.11 to 0.05)	0.45
Age maximum height reached ≥15 years †	0.49 (–0.59 to 1.57)	0.37	0.97 (–0.17 to 2.12)	0.097	–0.33 (–0.72 to 0.05)	0.091	–0.19 (–0.47 to 0.08)	0.16
Height change after 15 years (cm)	0.16 (–0.11 to 0.42)	0.25	0.20 (–0.09 to 0.49)	0.17	–0.06 (–0.16 to 0.04)	0.22	–0.03 (–0.10 to 0.04)	0.41

*At first assessment. Adjusted for age at measurement

† Estimated for treated women only.

The anthropometric variables that were significantly associated with any one or more of the four mammographic outcome variables (see **Table 8.3**) included bone age minus chronological age; and height, weight and BMI at first assessment, adjusted for age at measurement. Bone age minus chronological age and height at first assessment (adjusted for age at assessment) were negatively associated with dense and percent density, and positively associated with non-dense and total breast areas. Weight and BMI at first assessment (adjusted for age at assessment) were negatively associated with percent density, and positively associated with non-dense and total breast areas. A weak negative association was observed between dense area and weight at first assessment (p=0.059).

The association between height at first assessment with each of the mammographic measures remained after further adjustment for current height. In contrast, the associations between weight at first assessment (adjusted for age at measurement) and dense area, percent density and total breast area were no longer statistically significant after adjusting for current BMI (p-values 0.51, 0.10, and 0.06, respectively). The association with non-dense area remained (p=0.04). Similarly, the association between BMI at first clinic visit and dense area, percent density, total breast area and non-dense area were no longer statistically significant after adjusting for current BMI (p-values p=0.97, 0.35, 0.06, and 0.13 respectively).

None of the other anthropometric variables summarised in **Table 8.3** were significantly associated with the mammographic outcome variables, although birthweight and age when maximum height was attained appear to be potentially relevant covariates based on the size of the coefficients and the confidence intervals for some mammographic measures (e.g. dense area).

8.3.3 The influence of anthropometric parameters on the association between treatment and mammographic density

The childhood anthropometric variables available for treated and untreated women (birthweight, birth-length, bone age minus chronological age and height, weight and BMI at first assessment) were included in the model for treatment effect on dense area and each of

the other mammographic measures to assess the degree to which they might influence these associations. The following section describes these analyses separately for dense area, percent density, non-dense area, and total breast area.

8.3.3.1 Dense area

The following section describes the treatment coefficients for dense area (sqrt) adjusted for age and BMI for each of birthweight, birth-length, bone age minus chronological age and height, weight and BMI at first assessment. These analyses were performed in a restricted sample using only those for whom data for each of the anthropometric variables were available. The univariable analyses using restricted samples differ slightly from the larger sample analyses reported above in **Table 8.3**. The variables that remained in the final model in Chapter 7 were included in the regressions.

Dense area and birthweight and birth-length

In the restricted sample of 184 women for whom birthweight data were available, birthweight, when added to the regression (with age and BMI), enhanced the difference in dense area between treated and untreated women, from –0.50 (95%.CI: –1.07 to 0.07; p=0.09) to a coefficient of –0.57 (95% CI: –1.14 to –0.002; p=0.05). However, this was not the case for birth-length in the analysis of women for whom birth-length data were available (n=106).

In the restricted sample of 106 women for whom birth-length were available, the age and BMI adjusted regression coefficient for treatment on dense area did not change [–0.85 (95% CI: –1.62 to –0.09; p=0.03) to –0.84 (95% CI: –1.61, –0.07; p=0.032)] with the addition of birth-length to the regression equation.

Dense area and bone age

The difference between bone age and chronological age is a measure of bone maturity. When adjusted for age at measurement (chronological age), bone age minus chronological age

increases the difference observed between treated and untreated in dense area as demonstrated by the larger negative coefficients. The age and BMI adjusted regression coefficient for treatment effect on dense area in the restricted sample for whom bone age data is available (n=237) changed from –0.40 (95% CI: –0.88 to 0.08; p=0.10) to –0.52 (95% CI: –1.01 to –0.03; p=0.04) with the addition of bone age minus chronological age.

Dense area and height at first assessment

When adjusted for age at measurement (chronological age), height at first assessment reduced the difference observed between treated and untreated as demonstrated by the lower negative coefficients. In the restricted sample of 239 women, height at first assessment, when added to the regression along with age and BMI, reduced the difference in dense area between treated and untreated women, from –0.43 (95% CI: –0.92 to 0.05; p=0.080) to –0.33 (95% CI: –0.85, 0.18; p=0.21).

Dense area and weight at first assessment

When adjusted for age at measurement (chronological age), weight at first assessment (adjusted for age at assessment) did not alter the difference in dense area between treated and untreated. The age and BMI adjusted regression coefficient for treatment on dense area changed from –0.49 (95% CI: –1.00 to 0.01; p=0.055) to –0.49 (95% CI: –1.00 to 0.02; p=0.059) with the addition of weight at first assessment to the regression equation (n=224).

Dense area and BMI at first assessment

When adjusted for age at measurement (chronological age), BMI at first assessment did not affect the difference observed between treated and women. The age and BMI adjusted regression coefficient for treatment on dense area changed from –0.49 (95% CI: –1.00 to 0.01; p=0.055) to –0.50 (95% CI: –1.01 to 0.01; p=0.053) with the addition of BMI at first assessment to the regression equation (n=224).

Bone age, height at first assessment and birthweight

When height at first assessment was added to the regression for treatment effect on dense area adjusted for age, BMI and bone age minus chronological age (n=234) (further adjusted for age at bone age measurement) the regression changed minimally from -0.52 (95% CI: -1.01 to -0.02; p=0.040) to -0.56 (95% CI: -1.14 to 0.02; p=0.059).

Adding birthweight to the equation (restricted to n=183 for whom birthweight data were available) containing age, BMI and bone age minus chronological age (adjusted for age at measurement) changed the treatment coefficient for dense area (sqrt) from –0.65 (95% CI: –1.22 to –0.07; p=0.028) to –0.70 (95% CI: –1.27, –0.13; p=0.017).

8.3.3.2 Percent density

The following section describes the treatment coefficients for percent density (sqrt) adjusted for age, BMI and number of livebirths for each of birthweight, birth-length, bone age minus chronological age and height, weight and BMI at first assessment. These analyses were performed in a restricted sample using only those for whom data for each of the anthropometric variables were available.

Percent density, birthweight and birth-length

In the restricted sample of 184 women for whom birthweight data were available, birthweight, when added to the regression (along with age, BMI and number of livebirths), enhanced the difference in percent density between treated and untreated women, from –0.55 (95% CI: –1.06 to –0.04) (p=0.034) to a coefficient of –0.62 (95% CI: –1.13 to –0.12; p=0.016). However, this was not the case for birth-length in the analysis of women for whom birth-length data were available (n=106). The age, BMI and number of livebirths adjusted regression coefficient for treatment on percent density did not change [–0.79 (95% CI: –1.47 to –0.11; p=0.024) to –0.79 (95% CI: –1.48, –0.11; p=0.024)] with the addition of birth-length to the regression equation.

Percent density and bone age

When adjusted for age at measurement (chronological age), bone age minus chronological age increased the difference observed between treated and untreated in percent density as demonstrated by the larger negative coefficients. The regression coefficient for treatment effect on percent density in the restricted sample (n=237) changes from –0.40 (95% CI: –0.84 to 0.05; p=0.081) to –0.51 (95% CI: –0.96 to –0.05; p=0.028).

Percent density and height at first assessment

In the restricted sample of 237 women, height at first assessment, when added to the regression, reduced the difference in age, BMI and number of livebirths adjusted percent density (sqrt) between treated and untreated women, from –0.41 (95% CI: –0.85 to 0.04; p=0.076) to –0.29 (–0.77, 0.19; p=0.23).

Percent density and weight at first assessment

When adjusted for age at measurement (chronological age), weight at first assessment (adjusted for age at assessment) the difference in percent density between treated and untreated did not change. The age, BMI and livebirths adjusted regression coefficient for treatment on percent density changed from –0.45 (95% CI: –0.92 to 0.01; p=0.056) to –0.41 (95% CI: –0.88 to 0.06; p=0.087 with the addition of weight at first assessment to the regression equation.

Percent density and BMI at first assessment

BMI at first assessment (adjusted for age at assessment) did not significantly affect the difference in percent density between treated and untreated. The age, BMI and livebirths adjusted regression coefficient for treatment on percent density changed from –0.45 (95% CI: –0.92 to 0.01; p=0.056) to –0.46 (95% CI: –0.93 to 0.004; p=0.052) with the addition of BMI at first assessment to the regression equation.

Bone age, height at first assessment and birthweight

When height at first assessment was added to the regression for treatment effect on dense area adjusted for age, BMI and bone age minus chronological age (adjusted for age at measurement) (n=234), the regression coefficient did not change [-0.51 (95% CI: -0.97 to -0.05; p=0.028) to -0.51 (-1.05 to 0.03; p=0.062)].

Adding birthweight to the model for percent density (sqrt) adjusted for age, BMI, number of livebirths and bone age minus chronological age (adjusted for age at measurement) (restricted to n=183 for whom birthweight data were available) changed the treatment coefficient from –0.67 (95% CI: –1.18 to –0.16; p=0.011) to –0.73 (95% CI: –1.23 to –0.22; p=0.005).

8.3.3.3 Non-dense area

The following section describes the treatment coefficients for non-dense area (log) adjusted for age, BMI and number of livebirths for each of birthweight, birth-length, bone age minus chronological age and height, weight and BMI at first assessment. These analyses were performed in a restricted sample using only those for whom data for each of the anthropometric variables were available.

Non-dense area and birthweight and birth-length

In the restricted sample of 184 women for whom birthweight data were available, birthweight, when added to the regression, did not dramatically influence the regression coefficient for treatment on non-dense area; [changed from 0.12 (95% CI: –0.03 to 0.27; p=0.12) to 0.14 (0.08) (95% CI: –0.02 to –0.29; p=0.082)].

Similarly for birth-length (n=106) the age, BMI and livebirths adjusted regression coefficient for treatment on non-dense area changed minimally from 0.15 (95% CI: –0.07 to 0.37; p=0.18) to 0.16 (95% CI:–0.06 to –0.38; p=0.16) with the addition of birth-length to the regression equation.

Non-dense area and bone age

When adjusted for age at measurement (chronological age), bone age minus chronological age did not significantly influence the coefficient for treatment effect on non-dense area in the restricted sub-sample of participants for whom bone age data were available (n=237). The regression coefficient for treatment effect on non-dense area (adjusted for age, BMI and number of livebirths) in the restricted sample changed from 0.06 (95% CI: –0.08 to 0.19; p=0.42) to 0.08 (95% CI: –0.06 to 0.22; p=0.26).

Non-dense area and height, weight and BMI at first assessment

When adjusted for age at measurement (chronological age), height, weight and BMI at first assessment did not alter the regression coefficient for treatment on non-density in any meaningful way in the restricted samples of participants for whom data were available for each of these variables.

8.3.3.4 Total breast area

Restricted analysis within the samples for whom data were available for each of birthweight, birth-length, bone age; and height, weight and BMI at first assessment, did not affect the age and BMI adjusted regression coefficient for treatment once they were added to the regression. For instance, adding birthweight to the restricted sample of n=184 did not change the age and BMI adjusted coefficient for treatment on total breast area: from 0.05 (95% CI: –0.05 to 0.15; p=0.35) to 0.05 (95% CI: –0.05 to 0.16; p=0.31). This was similar for birth-length, from 0.03 (95% CI: –0.11 to 0.17; p=0.65) to 0.03 (95% CI: –0.11 to 0.17; p=0.65); bone age from 0.02 (95% CI: –0.08 to 0.11; p=0.75) to 0.02 (95% CI: –0.07 to 0.11; p=0.68), height at first assessment from 0.009 (95% CI: –0.08 to 0.10; p=0.85) to 0.0001(95% CI: –0.10 to 0.10; p=0.99).

8.4 Discussion

This final chapter aimed to assess the degree to which a number of pre-treatment childhood anthropometric measures such as childhood height, BMI and bone age might influence the association observed between treatment and mammographic density that was reported in Chapter 7. The study also aimed to examine the association between a number of childhood anthropometric measures that might have been influenced by treatment (e.g. BMI change, age at which maximum height was attained) on each of the mammographic measures for treated women only.

The pre-treatment variables were investigated because they were possible confounders in the association between mammographic dense area as an adult and treatment with high-dose estrogens in adolescent girls. If any of these anthropometric variables were independently associated with mammographic density and they were found to differ between treated and untreated women, they might change the magnitude of the difference in the separate independent treatment effect on mammographic density in treated and untreated women.

The post-treatment variables were investigated because they were possible mediators in the association between mammographic dense area as an adult and treatment with high-dose estrogens in adolescent girls. These variables were investigated in treated women only.

One of the reasons untreated girls were not treated with high-dose estrogens was that their bone age indicated insufficient growth potential to warrant treatment[25]. As expected, bone maturity at a given chronological age was greater in untreated girls compared with treated girls. It is possible that this parameter confounded the association observed between mammographic density and treatment as they were significantly associated with both treatment and outcome as demonstrated by a univariable analysis. However, adjusting for bone age minus chronological age (further adjusted for age at measurement) increased the difference in square root dense area between treated and untreated women. A similar effect was observed with percent density, which reached significance. Bone age minus

chronological age (adjusted for age at measurement) acted as a negative confounder because adjusting for it resulted in a strengthening of the association between treatment with high-dose estrogens and dense area, and percent density.

No published studies have examined the association between bone age in adolescence and mammographic density. Bone age has been used as a surrogate marker of growth plate senescence[483]. Girls whose bone age indicates little growth potential are also more advanced pubertally than those with a lower bone age at the same age or measurement[484]. The results suggest a strong negative univariable association between bone age minus chronological age and dense area and percent density, and a positive association with non-dense area and total breast area. This translates to increasing mammographic density with lower skeletal maturity or a later age of puberty, consistent with the established finding that early age of menarche is associated with reduced mammographic density. If treated girls had less advanced bone maturity, as indicated by the difference in bone age minus chronological age between treated and untreated girls measured at a mean age of 12 years, then it would be expected that they would have a higher mammographic density, independent of the effect of treatment. Adjusting for bone age minus chronological age would be expected to reduce this pre-treatment component to mammographic density. Since treated women had lower mean dense area compared with untreated women, this negative difference would be widened when adjusted for bone age minus chronological age; as observed. Added to this is the possibility that treatment accelerated puberty, resulting in less net growth of dense area.

It is interesting to note that Luo et al.(2003)[485] found faster linear growth during infancy and childhood (up to age 8) to be associated with earlier peak height velocity (PHV) during adolescence and less height gain between ages 8 and 18. This is consistent with a study by Biro et al. (2001)[486] which could suggest that untreated children were taller at younger years, and therefore reached skeletal maturity and consequently pubertal maturity earlier (PHV occurs ~12 months before menarche).

The other anthropometric variables that were significantly associated with all of the mammographic measures (dense area, percent density, total breast area and non-dense area)

included childhood height, weight and BMI at first assessment; however the associations with the latter two did not remain after adjustment for current BMI. They also did not change the coefficient for treatment for each of the mammographic density measures when included in the full multivariable models.

Height at first assessment, adjusted for age at measurement was negatively associated with dense area and percent density, and positively with total breast area and non-dense area. While statistically significant, the associations were small, and remained after adjustment for current height. The direction of the association is consistent with McCormack et al. (2003)[406] who found height at 11 years in girls had a negative association with higher Wolfe grade density OR 0.89 (95% CI: 0.80 to 1.00; p=0.04) (n=1090). They interestingly, observed an opposing association for children 2 years of age when followed-up; adjusted OR 1.13 (95% CI: 1.01 to 1.26; p=0.03) (n=1033). They found no significant association between height at ages 4, 7, or 15 years and Wolfe grade. In contrast, Sellers et al. (2007)[419] observed a positive association between height at ages 7 (p<0.001), 12 (p<0.001), and 18 years (p<0.001) with percent density (n=1893). In this PhD study, adjusting for height at first assessment (further adjusted for age at measurement) reduced the difference in square root dense area between treated and untreated women; however it had no effect when bone age minus chronological age remained in the model. A similar result occurred for percent density.

While birthweight was not statistically significantly associated with any of the mammographic measures, the confidence intervals and p-values suggest a positive association with dense area and to a lesser extent, percent density. This is consistent with a study that also examined the association between birthweight and density measured using a computer assisted technique by Cerhan et al. (2005)[431]. However, no association was observed in three studies using Wolfe grade as the measure of mammographic density[360, 406, 433]. Luo et al. (2003)[485] in their longitudinal study found greater birthweight and length to be associated with later peak height velocity in adolescence. If later peak height velocity, and therefore menarche (peak height velocity occurs ~1 year before menarche), was associated with higher mammographic density then it would be expected that greater birthweight and birth-length would also be positively associated with mammographic density.

When birthweight was added to the regression equation along with age, BMI and bone age minus chronological age, the difference in dense area (sqrt) between treated and untreated women increased. A similar effect was observed with percent density (with additional adjustment of number of livebirths). Birth-length did not have any effect when added to the regression model.

This chapter also aimed to examine the influence of anthropometric variables that might have been influenced by treatment e.g. (age at maximum height, and weight change following treatment). It is possible that changes to these variables following treatment mediate the reduced mammographic density observed in treated women compared with untreated women in Chapter 7, age at maximum height or growth beyond 15 years, were not associated with any of the mammographic measures for treated women, though numbers were small in the sub-group analysis. However, these findings are supported by Sellers and co-investigators in their study with larger numbers (n=1298)[419]. They found no association between age at which participants stopped getting taller and mammographic density measured by a similar computer assisted thresholding technique.

Weight gain following treatment might suggest a greater degree of estrogen responsiveness compared to those whose weight did not change. However weight gain one year after commencement of treatment was not associated with any of the mammographic measures in the univariable analysis, and even less so in the multivariable analysis. This is in contrast to the findings of McCormack et al. (2003)[406]. In their study, increases in BMI during any period up to age 43 years were associated with reduced odds of a greater Wolfe grade, with larger inverse associations in the preadolescent years (7–11 years).

Strengths of the study include the use of medical record data for childhood anthropometric data and the inclusion of bone age data which is not normally available in child health records. Bone age data were collected close to the time of height, weight and BMI measurements at first assessment providing more consistency in these growth related exposures.

On the other hand, a limitation of the study was the lack of medical record data for some women in the study. This reduced the sample size for many of the sub-group analyses.

Not all women provided consent to access their records, and not all records were available. Also of those records that were available, not all data were recorded for all (e.g. birth-length). In addition, growth velocity, which might have differed between treated and untreated girls (before treatment and as a result of treatment), could not be measured. McCormack et al. (2003)[406] found a positive yet statistically insignificant greater odds of having a higher Wolfe grade with increasing height velocities at ages 11–15 years (p=0.08) but to a lesser extent in 7–11 years (p=0.96) (n=1030).

Another limitation is the potential inaccuracy of bone age data. According to Drop et al. (1998)[44] the techniques of bone age assessment are subjective, and that this subjectivity is reflected in the inter- and intra-rater variability. The technique used in this study cohort was the Greulich-Pyle atlas for bone age assessment. Between 1959 and 1975, for all tall girls, the bone age of the hand-wrist was assessed by one observer. The mean intra-observer difference in assessments was calculated to be 1.1 months[60].

Self-reported height data at follow-up is a likely source of measurement error. However, according to Spencer et al. (2002)[487], taller women report height more reliably than shorter women. In addition, height is a prominent characteristic for women with a history of assessment or treatment for tall stature[77]. Similarly, age at maximum height is crudely measured in this study and is likely to be a source of measurement error.

8.5 Conclusion

The aims of this chapter were 1) to examine the degree to which a number of pre-treatment childhood anthropometric measures influenced the association observed between treatment and mammographic density; and 2) to examine the association between a number of post-treatment childhood anthropometric measures that might have been influenced by treatment (e.g. weight and BMI change, and age at which maximum height was attained) on each of the mammographic measures for treated women only.

Birthweight and bone age minus chronological age and height, weight and BMI at first assessment (adjusted for age at assessment) all showed univariable relationships with mammographic density of which only bone age minus chronological age and birthweight remained in the larger model for treatment effect on dense area and percent density. These two variables did not reduce the treatment effect on dense area and percent density, rather they increased it. In treated women, weight and BMI change following treatment and age which maximum height was attained were not associated with any of the mammographic measures after adjustment, and are unlikely to have had a mediating effect on the association between dense area and high-dose estrogen exposure in adolescent girls.

Box 8.1: Summary of key chapter findings.

KEY FINDINGS: CHAPTER 8

- Birthweight and bone maturity at first assessment (measured as bone age minus chronological age) and height, weight and BMI at first assessment (adjusted for age at assessment) all showed univariable relationships with mammographic density.
- Of the above variables, only bone age minus chronological age and birthweight remained in the larger model for treatment effect on dense area and percent density.
- Bone age minus chronological age and birthweight strengthened the treatment effect on dense area and percent density.
- In treated women, weight and BMI change following treatment and age which maximum height was attained were not associated with any of the mammographic measures after adjustment, and are unlikely to have had a mediating effect on the association between dense area or percent density and high-dose estrogen exposure in adolescent girls.

PART D

Chapter 9: Conclusion

References

Appendices

9: CONCLUSION

The aim of this research was to examine the short- and long-term effects of adolescent exposure to high-dose estrogens on the breast. Chapter 4 explored the short-term side effects on the breast in a cohort of Australian women who were treated with high-dose estrogens in adolescence. Self-reported breast-related side effects of treatment included breast lumps, galactorrhea, breast pain, dry cracked or bleeding nipples and increased pigmentation of the nipple and areolae. Dry cracked nipples, not reported previously in the literature on treatment for tall stature, might be associated with nipple hyperkeratosis which is known to be associated with estrogen treatment in humans[235] and animals[236].

Side effects most frequently reported were increased pigmentation of the nipple (33.7%) followed by breast pain (8%). These appear to be similar in frequency to that observed elsewhere[22, 23, 29]. Also, more diethylstilbestrol (DES) treated women seemed to have experienced these side effects compared with ethinyl estradiol (EE) treated women. The observation of breast pain may be significant to breast cancer risk as Crandall et al. (2009)[237] observed an increased risk in women who had experienced new-onset breast tenderness following hormone replacement therapy (HRT) use.

Chapter 4 also explored the risk of having had a breast biopsy and breast surgery (including lumpectomy) in treated and untreated women. Biopsies and surgery were more likely to be remembered by participants compared with reporting a diagnosis of benign breast disease or a breast lump, and evidence of a clinical investigation was considered important for a diagnosis. No significant difference was observed in having had these procedures between treated and untreated women. While the number of breast biopsies may not accurately represent the prevalence of benign breast disease in this study cohort, if treated women had more cases of breast disease than untreated women it would be expected that they would have had more breast biopsies and breast surgery for lumpectomy (excluding cancer) for a given age.

Treated women were no more likely to have had a diagnosis of breast cancer than untreated women. While the cohort size was too small to pick up sufficient cases for a reliable assessment of the level of breast cancer risk associated with high-dose estrogen treatment in tall girls, it provided some indication of the difference in the rates between the two groups of women. If breast cancer risk was hugely increased in treated women it might have been observed with the current sample size. Future studies should consider a longer follow-up period to include women who are at an age when the incidence of breast cancer is greater, and a larger sample size.

Chapter 5 examined the long-term effects of treatment on lactation, in particular, breastfeeding commencement and duration. This study found no meaningful differences in breastfeeding commencement or breastfeeding duration, between women who were either treated or untreated with high-dose estrogens during adolescence.

The reasons for stopping breastfeeding were similar between treated and untreated women further validating the findings on breastfeeding initiation and duration. Treated women were no more likely to have stopped breastfeeding because of a lack of milk, nipple trauma or pain, or mastitis. More treated than untreated women reported stopping because their breasts did not engorge or fill with milk but the numbers were small for both groups.

Women with insufficient glandular tissue of the breast are known to suffer from lactation failure and a lack of breast enlargement during pregnancy[230, 258]. Complaints of flat chestedness by girls treated with estrogens for tall stature have been reported[23, 41]. It is possible that the reduced IGF-I levels observed with treatment[11] could result in reduced glandular tissue in the developing breast and therefore increase the risk of breast hypoplasia and consequently a lack of breast enlargement during pregnancy and lactation insufficiency. However this study found that women treated with estrogen during adolescence for tall stature were no more likely to report a lack of breast enlargement during pregnancy or lactation insufficiency than untreated women. In addition, a lack of breast engorgement or filling of the breasts as a reason for stopping breastfeeding did not seem to be associated with breast hypoplasia because most of the women reporting a lack of breast engorgement or filling of the breasts reported an increase in breast size during pregnancy. Chapters 7 and 8

examined the effect of treatment on total breast area, and while treated women had less mean total breast area than untreated women, this difference was not statistically significant.

To better investigate long-term breast effects, mammographic density, a well established risk factor of breast cancer, was investigated in Chapters 7 and 8. The Australian cohort of women who were assessed for tall stature in adolescence and either treated or not treated were followed up a second time. Women's mammograms were collected and mammographic density measured using a computer-assisted thresholding technique. This study found that women treated with estrogen for tall stature in adolescence had a significantly lower mean dense area than women who were assessed for tall stature but untreated. Treated women had lower percent density, and slightly greater adjusted mean non-dense area compared with untreated women but these differences were not statistically significant.

Chapter 8 examined the degree to which a number of pre-treatment growth parameters influenced the association observed between treatment and mammographic density. It also explored the association between a number of adolescent anthropometric measures that might have been influenced by treatment (e.g. weight and BMI change, and age at which maximum height was attained) on each of the mammographic measures for treated women only. While birthweight and bone age minus chronological age and height, weight and BMI at first assessment (adjusted for age at assessment) all showed univariable relationships with mammographic density, only bone age minus chronological age and birthweight remained in the final model for treatment effect on dense area and percent density. These two variables did not reduce the treatment effect on dense area, rather they increased it.

There are a number of limitations of this study. Treated women (mean age 39.8 years) were asked to recall side effects of treatment that occurred in adolescence. While recall might have been biased towards those side effects that were troublesome, the side effects that were recalled are likely to be accurate. Reassuringly, the type and frequency of side effects reported by the women in the study were found to be consistent with those in published case-series reports.

Recall error is also relevant to breastfeeding history. Women were asked to recall their breastfeeding history on average 10.7 years (range 0.4–33 years) after breastfeeding their first infant. However, a review of 11 studies that examined the reliability and validity of breastfeeding recall data found maternal recall to be a valid and reliable estimate of breastfeeding initiation and duration, but was less satisfactory for exclusive breastfeeding[259].

The examination of mammographic density as a measure of breast cancer risk was a strength of this study. With respect to concerns about high-dose treatment during adolescence and future breast cancer risk, this study has shown that such treatment does not increase mammographic density. On the contrary, treated women in this study had an overall lower mean mammographic dense area than untreated women.

There are a number of limitations of the mammographic density measure in this study. It is a 2-dimensional measure that does not capture 'depth' of density and there is a degree of variability in the quality of the mammograms and subjectivity in its measurement. Yet, despite these shortcomings, studies consistently show an association between mammographic density, using the methods used in this study, and breast cancer risk. Boyd and colleagues suggest that every 4.06 cm^2 increase in total mammographic dense area is associated with an increase of 3% in breast cancer risk[36]. If true, our study would suggest that treated women were at a 3% reduced risk of breast cancer if all other risk factors remained the same.

This study suggests that hormone treatment in adolescence has had an effect many years after the discontinuation of treatment and that that hormonal exposures during the pubertal mammary development period have long lasting effects. The mechanism of this action is not clear.

The observed effects (and lack of effects) of adolescent exposure to high-dose estrogens observed in this study should not be generalised to all exogenous estrogen exposures that might occur during adolescence. Estrogen has a biphasic effect on some tissues[7] exerting a different action at low concentrations than at high concentrations. It may follow, therefore, that lower exogenous estrogen exposures during adolescence may have a

different effect on breast tissue and function than that observed with the high estrogen exposures in this study.

This is the first study to examine the long-term effects of high-dose estrogen exposure in adolescence on the breast. The retrospective cohort study adopted to examine these effects was the optimum design of choice. An RCT was not possible, and a case-control study design would not have been suitable, given that treatment is not common. While a retrospective design has its shortcomings (e.g. relies on medical records or recall for exposure measurement), it also has its strengths. This study design enabled an extended follow-up of the effects of an uncommon exposure and the examination of potential confounders in the data analysis.

While strength of study design and the exclusion of potential confounders as possibly explanatory factors are important in the determination of causality, other criteria need to be considered. Bradman Hill's widely used and cited list of causal criteria include, strength of association, consistency in findings across studies, temporality, biological gradient, plausibility, coherence, experimental evidence, specificity and analogy[488]. Strength of association should not necessarily be considered when interpreting causality. Rothman argues that weak associations can be causal and strong associations non-causal. In relation to the second criterion above, (consistency in findings across studies) this is the first study to have explored the association between treatment for tall stature and mammographic density as an adult. Additional studies would be helpful and may or may not add further support to the findings.

Temporality is important when considering causality. In relation to Hill's criteria of temporality, exposure is required to precede the outcome. It is possible that girls treated with high dose estrogens had lower mammographic density than untreated girls before treatment. This study cannot discount this possibility. In addition, pre-treatment factors associated with mammographic density later in life might have differed between the groups. The untreated participants were similarly tall as girls. The main reasons for not being treated were because the girl's estimated mature height or remaining growth potential at time of assessment did not warrant treatment. While it is possible that pre-treatment anthropometric differences may explain some of the effects on mammographic density, potential anthropometric confounders

were collected from the medical records and included in the analysis. They did not reduce the observed effect on mammographic density.

Biological gradient is not relevant to this study because dose of estrogen treatment was similar for all treated girls. Plausibility as a criterion of causality can be questioned. A causal association can exist without knowing the mechanism underpinning the association. None-the-less, the findings that girls treated with high dose estrogens had lower mammographic dense tissue than untreated girls could possibly be explained by the observations made elsewhere that IGF-I levels were reduced during treatment. IGF-I has been positively associated with mammographic density, and a reduction during puberty could possibly contribute to a reduction in mammographic density as an adult.

Specificity is not necessarily an important criterion of causality. Many factors do contribute to change in mammographic density. In terms of coherence with existing theory and knowledge, the association between mammographic density and exposure to high-dose estrogens as an adult was explored because there was a strong *a priori* reason for doing so. Hormone treatment as an adult is known to influence adult mammographic density and suggests that hormone treatment in adolescence might also have an effect. While Bradman Hill did not promote the use of his list of causal parameters to be used as a checklist, they can be useful when relevant to the association under investigation. On balance, the association between mammographic dense area and treatment with high-dose estrogens in adolescence appears likely to be causal, but replication of these findings in other studies, or alternative studies that explore the association between hormone treatment and mammographic density in adolescence would be needed to support the causal inferences made in this study.

As well as helping to address the concerns of treated women, the research provided a rare opportunity, internationally, to examine the long-term biological effects of this treatment. While this study found that women treated with high-dose estrogens for tall stature in this cohort experienced unpleasant side effects on the breast during treatment, it also provides some reassurance for these women that treatment does not appear to affect their ability to lactate or increase their risk of having breast disease requiring a breast biopsy or surgery, and is unlikely to increase their risk of breast cancer through mechanisms related to mammographic density. The study also has broad implications for our understanding of the

biology of breast development and for breast cancer research. It has shown us that exposure to sex hormones during adolescence can have a sustained effect on breast tissue as demonstrated by a reduction in mammographic dense tissue in adulthood.

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APPENDIX 1

Sample size calculation for breast cancer risk

APPENDIX 1

In Australia, the 20 year breast cancer prevalence rate for women 35-39 years of age in 2006 was 1.6%. This prevalence rate is based on the number of surviving persons who received a breast cancer diagnosis in the last 20 years[1].

The mean ages of women in the Tall Girls Study were 39.7 years (treated) and 37.7 years (untreated) at the time that they were asked about their breast cancer history. It would be expected, assuming there was no increased or decreased risk of breast cancer in these women and based on the population prevalence rates, that at least 1.6% of women would have had a diagnosis of breast cancer previously.

The known sample size for treated and untreated women in the cohort is 371 and 409, respectively. Using the sampsi command in Stata, if more treated women have had a previous diagnosis of breast cancer than untreated women, then at least 6% of treated women would need to have had breast cancer for the study to have 80% power to detect a significant difference between the two groups (assuming the proportion of untreated women is 1.6%).

Stata command: sampsi .06 .016, alpha (.05) power (.8)

To observe a 20% relative increase in risk in treated women

P=proportion
P treated / P untreated =1.20
P treated =1.20 x P untreated
P treated = 1.20 x 1.6/100 = 1.92/100

The difference in the absolute scale is 1.92/100 -1.6/100 = 0.32/100 or 0.32%
Stata command: sampsi .0192 .016, alpha (.05) power (.8)

We would need a sample size of 27,000 for each of the treated and untreated groups.

To observe a RR of 2.0 or relative increase of 100%:

P treated/P untreated =2.00
P treated= 2.00 x P untreated
P treated = 2.00 x 1.6/100= 3.2/100

The difference in the absolute scale is 3.2/100 – 1.6/100 = 1.6/100 or 1.6%
Stata command: sampsi .032 .016, alpha (.05) power (0.8)

We would need 1558 for each treated and untreated.

Source: http://distance.jhsph.edu/statr2/heart/modules/resources/pdf/statr2-sec3c_6.pdf

1. Australian Institute of Health and Welfare & National Breast Cancer Centre 2006. Breast cancer in Australia: an overview, 2006. Cancer series no. 34. Cat. no. CAN 29 Canberra: AIHW.

Appendix 1: Sample size calculation for breast cancer risk

APPENDIX 2

Follow-up 1 study invitation letter, information brochure and consent form

Appendix 2: Follow-up 1 invitation letter, study information sheet and consent form.

APPENDIX 2

October 22, 2002

Dear «First_name»,

We are writing to ask for your help in our study that aims to find out about the long-term effects of hormone treatment to reduce the adult height of tall girls. Treatment with oestrogens to reduce the adult height of tall girls has been available in Australia and in other countries since the 1950s but there have been few follow-up studies. This research, which includes treated **and untreated** tall girls, will make an important contribution to our understanding of the health and psychosocial issues that are important to tall women and girls. The National Health and Medical Research Council has funded the study which is being conducted by a team of researchers at the Centre for the Study of Mothers' & Children's Health, La Trobe University and the Royal Children's Hospital.

Your name was obtained from: a list of names provided to us by the late Dr XXX, the mailing list of Tall Girls Inc., or from the details you gave us when you contacted us and expressed an interest in this study. Dr XXX gave the study his full support before his death in late 2000. We are seeking your help even if you never had treatment for tall stature. This will allow us to compare outcomes in tall girls who were treated with those who were not treated. Eligible women are those who consulted a doctor about their growth and had x-ray assessment to predict their expected mature height.

Participation in the study will involve answering the enclosed questionnaire asking about your health and wellbeing, and satisfaction with treatment or the decision not to have treatment. In addition, we would like to ask you to complete a telephone interview about your medical and reproductive history, at a time convenient to you, in the next few months. We also ask you to give us permission to obtain information from your medical records. The enclosed information sheet and consent form give further details.

Participation in the study is entirely voluntary. If you choose to participate, you are free to withdraw from the study at any time. The information you provide will be kept confidential. The results of the study will not be published in any way that would allow you to be identified.

The enclosed questionnaire will take about 30 minutes to complete. We would be grateful if you could complete it and return it with the informed consent form in the enclosed reply paid envelope as soon as possible. If we do not hear from you in the next few weeks we may contact you again to check that you have received this letter. It is very important for us to have as many replies as possible. We would like to hear from women with good health as well as those with health problems, and from women with a range of views about the medical assessment and treatment of tall stature. If you have any questions about the study, please feel free to contact Jo Rayner or Penny Jones, the study's research nurses, on (03) 8341 8582.

We look forward to hearing from you soon.

Yours sincerely

Dr Alison Venn
Centre for the Study of Mothers' & Children's Health

Professor George Werther
Royal Children's Hospital

«ID_number»

Appendix 2: Follow-up 1 invitation letter, study information sheet and consent form.

Long-term health and psychosocial effects of hormone treatment to reduce the adult height of tall girls

Study information sheet

What is the study about?

This study aims to find out about the long-term health and psychosocial effects of hormone treatment to reduce the adult height of tall girls.

Who is eligible to participate?

You are eligible to participate if you had a medical assessment of your expected mature height before 1990. Assessment would have included an x-ray of your hand and wrist as well as measures of your height when you were in your adolescence. We want to follow-up tall girls who were not treated, as well as those who received hormone treatment to reduce their adult height.

How did you get my name?

Your name was obtained from one or more of the following:

- A list of patient names provided by **XXX** prior to his death in late 2000.
- Details you gave us when you contacted us previously to register your interest in the study.
- The mailing list of Tall Girls Inc.

What exactly do you want me to do?

- Answer a postal questionnaire about yourself, your health and satisfaction with your treatment decision
- Measure your height and weight
- Complete a telephone interview about your medical and reproductive history
- Give us permission to access your medical records so that we can confirm details about your height assessment and, if applicable, your treatment for tall stature.

In the coming months you may also be asked to participate in an in-depth individual interview about your experiences of being tall and assessed or treated. However, only a small number of women will be asked to do this and you do not have to agree to such an interview now.

Do I have to participate?

Participation in the study is entirely voluntary. You are free to withdraw from the study at any time. You are also free to not answer any particular question. If you do not wish to participate in the study, please let us know and we will not contact you further.

What's in it for me?

This study will be the first study to examine the long-term outcomes for women who had an assessment or treatment for tall stature when they were girls. You may find that you experience some benefit from being able to give an account of your experiences and health. The study will benefit other women and the medical and research community by increasing our understanding of the effects of oestrogen treatments during puberty. It will also help us to evaluate the effectiveness of treatment for tall stature. The study will increase our knowledge of how being tall, or being treated for tall stature, has affected women's lives. It is likely that the findings will be helpful to clinicians in addressing the concerns of tall girls and their families who present for clinical assessment, even though this now occurs relatively infrequently.

Appendix 2: Follow-up 1 invitation letter, study information sheet and consent form.

What about my privacy?

The information you provide will be kept confidential. The data collected will be used only for the purposes of this study. Your answers to the questionnaire and telephone interview will be coded and entered onto a computer without your name. The telephone interviewer will only know your first name. They will not know your full name or treament status. The study results will be published in a way that will not allow you to be identified. The study has been approved by the ethics committees of La Trobe University and the Royal Children's Hospital.

Who is doing the research?

The study is being conducted by a group of researchers led by Dr Alison Venn. Professor Judith Lumley and Dr Priscilla Pyett (Centre for the Study of Mothers' & Children's Health, La Trobe University) and Professor George Werther (Centre for Hormone Research, Royal Children's Hospital Research Institute) are the other researchers involved in the study. Their collective expertise is in epidemiology, health sociology and paediatric endocrinology. Ms Penny Jones and Ms Jo Rayner are the study's research nurses and Ms Fiona Bruinsma is the data manager.

Who is funding the study?

The study is funded by a grant from the National Health and Medical Research Council.

Can I be told about the results?

We expect to have results in about two years' time. If you would like to hear about the results, please indicate this in the postal questionnaire. You can follow the study's progress on our website at http://www.latrobe.edu.au/csmch/tallgirls/

Who do I contact if I have questions or concerns?

If you have any concerns or questions or would like more information contact Jo, Penny or Fiona
Centre for the Study of Mothers' & Children's Health,
251 Faraday Street, Carlton VIC 3053
Phone (03) 8341 8583, fax (03) 8341 8555
Email tgstudy@latrobe.edu.au
If at any time you have any concerns about your involvement in the study that the researcher has not been able to answer to your satisfaction, you may contact the Ethics Liaison Officer, Human Ethics Committee, La Trobe University, Bundoora, VIC 3083 (ph (03) 9479 1443, email: humanethics@latrobe.edu.au

Appendix 2: Follow-up 1 invitation letter, study information sheet and consent form.

Study ID number: __ __ __ __ __

Centre for the Study of Mothers' and Children's Health

LA TROBE UNIVERSITY

CONSENT FORM

The long-term health and psychosocial effects of oestrogen treatment to reduce the adult height of tall girls.

Chief Investigators:

DR ALISON VENN
Centre for the Study of Mothers' & Children's Health,
La Trobe University and Menzies Centre for Population Health Research, University of Tasmania

PROFESSOR JUDITH LUMLEY & DR PRISCILLA PYETT
Centre for the Study of Mothers' & Children's Health, La Trobe University

PROFESSOR GEORGE WERTHER
Centre for Hormone Research, Royal Children's Hospital Research Institute

(A) Consent to participate

I have read and understood the information on the study information sheet. Any questions I have asked have been answered to my satisfaction.

I agree to participate in the project by:

- filling in the questionnaire
- providing information on my current height and weight
- participating in the telephone interview

I realize I may withdraw at any time. I agree that research data provided by me or with my permission during the project may be included in a thesis, presented at conferences and published in journals on the condition that neither my name nor any other identifying information is used.

Name of participant .....

Signature ..... Date ...../...../.....

(B) Consent to obtain medical information

We would like to contact the doctor(s) who assessed and/or treated you for tall stature. We will, however, only do this with your permission. We are requesting access to your medical records so that we can confirm details about your height assessment and, if applicable, your treatment for tall stature. All the information we collect will be kept **confidential**. The information we collect will be **used solely for the purposes of this research study**.

Please provide us with the name and address(es) of the doctor or hospital who assessed or treated you for tall stature, and the year that you attended. If you are unsure of a doctor's name or address, please give us whatever information you do have.

Appendix 2: Follow-up 1 invitation letter, study information sheet and consent form.

(1) Dr or hospital.....Year attended .....

Address .....

.....State.....

Postcode.....

(2) Dr or hospital.....Year attended .....

Address .....

.....State.....

Postcode.....

Your name ..... Maiden/other surname(s) used .....

(print name)

Your signature ..... Date ...../...../.....

Appendix 2: Follow-up 1 invitation letter, study information sheet and consent form.

APPENDIX 3

Follow-up 1 postal questionnaire

CONFIDENTIAL

The Tall Girls Study

POSTAL QUESTIONNAIRE

We would be grateful if you would agree to take part in our study by answering this questionnaire. It asks questions about your experiences of being tall, being assessed and/or being treated for tall stature, as well as your health and medical history. It takes about 30 minutes to complete.

When completed please return it in the reply paid envelope to

Tall Girls Study
Reply Paid number 64593

Centre for the Study of Mothers' & Children's Health
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If you have any questions about the study
or would like assistance to complete this questionnaire over the telephone,
please call Penny Jones (03) 8341 8581 or Jo Rayner (03) 8341 8582.

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For office use only

ID__ __ __ __ __ __

Date sent ____/ ____/ ____

Date received ____/ ____/ ____

Checked by FB PJ JR Date checked / /

Please read below instructions

Please answer every question. Please complete the questionnaire by

(i) filling in the appropriate number in each box
eg. How tall are you now? [5] feet [1][1] inches OR

(ii) circling a number (1) OR

(iii) ticking a box [✓]

If you have any additional information please add comments near that question or at the end of the questionnaire.

SECTION A These questions ask about your general health

These questions ask for your views about your health, how you feel and how well you are able to do your usual activities.

Answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

A1 In general, would you say your health is

	[circle one number]
Excellent	1
Very good	2
Good	3
Fair	4
Poor	5

A2 Compared to one year ago, how would you rate your health in general now?

	[circle one number]
Much better now than one year ago	1
Somewhat better now than one year ago	2
About the same as one year ago	3
Somewhat worse now than one year ago	4
Much worse now than one year ago	5

Section A continued

A3 **The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?**

[circle one number on each line]

	ACTIVITIES	Yes, limited a lot	Yes, limited a little	No, not limited at all
(a)	Vigorous activities such as running, lifting heavy objects, participating in strenuous sports	1	2	3
(b)	Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	1	2	3
(c)	Lifting or carrying groceries	1	2	3
(d)	Climbing several flights of stairs	1	2	3
(e)	Climbing one flight of stairs	1	2	3
(f)	Bending, kneeling or stooping	1	2	3
(g)	Walking more than one kilometre	1	2	3
(h)	Walking half a kilometre	1	2	3
(i)	Walking 100 metres	1	2	3
(j)	Bathing or dressing yourself	1	2	3

A4 **During the <u>past 4 weeks</u>, have you had any of the following problems with your work or other regular daily activities <u>as a result of your physical health</u>?**

[circle one number on each line]

	YES	NO
(a) Cut down on the amount of time you spent on work or other activities	1	2
(b) Accomplished less than you would like	1	2
(c) Were limited in the kind of work or other activities	1	2
(d) Had difficulty performing the work or other activities (for example it took extra effort)	1	2

A5 **During the <u>past 4 weeks</u>, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?**

[circle one number on each line]

	YES	NO
(a) Cut down on the amount of time you spent on work or other activities	1	2
(b) Accomplished less than you would like	1	2
(c) Didn't do work or other activities as carefully as usual	1	2

A6 **During the <u>past 4 weeks</u>, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?**

[circle one number]

Not at all	1
Slightly	2
Moderately	3
Quite a bit	4
Extremely	5

Section A continued

A7 **How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>?**

[circle one number]

No bodily pain	1
Very mild	2
Mild	3
Moderate	4
Severe	5
Very severe	6

A8 **During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?**

[circle one number]

Not at all	1
A little bit	2
Moderately	3
Quite a bit	4
Extremely	5

A9 **These questions are about how you feel and how things have been with you <u>during the past 4 weeks</u>. For each question, please give the one answer that comes closest to the way you have been feeling.**

How much of the time during the <u>past 4 weeks</u> - *[circle one number on each line]*

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
(a) Did you feel full of life?	1	2	3	4	5	6
(b) Have you been a very nervous person?	1	2	3	4	5	6
(c) Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
(d) Have you felt calm and peaceful?	1	2	3	4	5	6
(e) Did you have a lot of energy?	1	2	3	4	5	6
(f) Have you felt down?	1	2	3	4	5	6
(g) Did you feel worn out?	1	2	3	4	5	6
(h) Have you been a happy person?	1	2	3	4	5	6
(i) Did you feel tired?	1	2	3	4	5	6

A10 **During the <u>past 4 weeks</u>, how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting with friends, relatives, etc)?**

[circle one number]

All of the time	1
Most of the time	2
Some of the time	3
A little of the time	4
None of the time	5

Section A continued

A11 How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
	[circle one number on each line]				
(a) I seem to get sick a little easier than other people	1	2	3	4	5
(b) I am as healthy as anybody I know	1	2	3	4	5
(c) I expect my health to get worse	1	2	3	4	5
(d) My health is excellent	1	2	3	4	5

SECTION B: These questions ask about the support you get from others

The following statements have been used by many people to describe how much support they get from other people. We would like to know whether you share any of these feelings and how strongly you feel about them, by circling a number according to whether you strongly agree, agree, disagree or strongly disagree with each one. If you are undecided, circle the column with this heading (3).

		Strongly agree	Agree	Undecided	Disagree	Strongly disagree
B1	People don't come to visit me as often as I would like.	1	2	3	4	5
B2	I find it easy to make friends.	1	2	3	4	5
B3	I often need help from other people but can't get it.	1	2	3	4	5
B4	I'm afraid of being left alone.	1	2	3	4	5
B5	I seem to have a lot of friends.	1	2	3	4	5
B6	I don't have anyone that I can confide in.	1	2	3	4	5
B7	The person who means most to me takes an interest in my affairs.	1	2	3	4	5
B8	There is someone who needs me as much as I need them.	1	2	3	4	5
B9	I don't have a very close friend.	1	2	3	4	5
B10	The person who means most to me doesn't spend time with me.	1	2	3	4	5
B11	I have no-one to lean on in times of trouble.	1	2	3	4	5
B12	I have someone to share good news with.	1	2	3	4	5
B13	There is someone who can always cheer me up when I'm down.	1	2	3	4	5
B14	I often feel very lonely.	1	2	3	4	5
B15	I feel there is something missing from my life.	1	2	3	4	5

SECTION C: These questions ask about your [illegible]

Please now measure your height following the instructions below and write down the result in C1. You might also prefer to have someone help you do this.

	SELF MEASUREMENT INSTRUCTIONS
1	This measurement needs to be done before 12 midday because our height decreases very minimally over the day. You need to be in bare feet and on a hard floor, without carpet, to do this measurement.
2	Take a firm picture frame or similar rectangular or square object.
3	Have a pencil ready and stand with your back to the wall as straight and stretched as possible, keeping your chin tucked in.
4	Place the picture frame edge against the wall above your head so that the frame is horizontal and projecting over your head.
5	Slide the frame down the wall until the horizontal edge is sitting on your head. While holding the frame in place, use your other hand to draw a horizontal mark on the wall immediately below the frame edge. This mark will represent your height.
6	Use a tape measure to measure from the floor to the mark and record this as your height.
7	Please repeat the whole process as a check.

C1 How tall are you without shoes on? *[Give fraction of inch or centimetre]*

eg. *5* feet *11* inches *½* OR *181.5* cm

☐ feet ☐☐ ☐ inches OR ☐☐☐ . ☐ cm

C2 How much do you currently weigh without clothes or shoes? ☐☐ stones ☐☐ pounds or ☐☐☐ . ☐ kg

C3 Do you have any of the following rare conditions that have been occasionally associated with tall stature? *[circle the number that applies]*

	YES	NO
Marfan's syndrome	1	2
Homocystinura (clotting problems)	1	2
Pituitary gigantism (acromegaly)	1	2
47XXX karyotype	1	2
Sotos' syndrome	1	2
Neurofibromatosis	1	2
Hyperthyroidism	1	2
Precocious puberty	1	2

Section C continued

C4 **In general, are you satisfied with your current height?**
[circle one number]

Yes	1
No	2
Undecided	3

C5a **Ideally, what height would you like to be?** ☐☐☐ feet inches or ☐☐☐ cm

C5b Why? .....

.....

.....

.....☐☐

C6a **In your opinion, what would be the ideal height for a woman?** ☐☐☐ feet inches or ☐☐☐ cm

C6b Why? .....

.....

.....

.....☐☐

C7a **In your opinion, what is the maximum acceptable height for a woman?** ☐☐☐ feet inches or ☐☐☐ cm

C7b Why? .....

.....

.....

.....☐☐

C8a **In your opinion, what is the ideal height for your partner?**
[circle one number]

Taller than me	1
Shorter than me	2
Same size as me	3
Don't have an opinion	4

C8b Why? .....

.....

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.....☐☐

Section C continued

The next few questions ask you to think about the advantages and disadvantages of being tall when you were young and as an adult.

C9a **Please complete the following two statements:**

"When I was growing up, the <u>best</u> things about being tall were

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.....☐☐

C9b **"When I was growing up, the <u>worst</u> things about being tall were**

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.....

.....☐☐

C10 **In my experience being a tall woman is an <u>advantage</u> because**
[circle one number on each line]

	Strongly agree	Agree	Undecided	Disagree	Strongly disagree
(a) It is easier to reach things	1	2	3	4	5
(b) I can see over people in a crowd	1	2	3	4	5
(c) It has helped my work/career options	1	2	3	4	5
(d) Tall women are considered more elegant	1	2	3	4	5
(e) Tall stature gives you natural authority	1	2	3	4	5
(f) Other women envy my height	1	2	3	4	5
(g) It has allowed me to excel in sport	1	2	3	4	5
(h) It has enabled me to have more equal relationships with men	1	2	3	4	5
(i) I can easily attract the attention of others	1	2	3	4	5

Section C continued

C11 In my experience being a tall woman is a disadvantage because

[circle one number on each line]

	Strongly agree	Agree	Undecided	Disagree	Strongly disagree
(a) Standard size furniture is a problem	1	2	3	4	5
(b) It is difficult to find clothes that are suitable	1	2	3	4	5
(c) People have unrealistic expectations of me	1	2	3	4	5
(d) Tall stature has limited my opportunities to achieve	1	2	3	4	5
(e) People make unwelcome comments about my height	1	2	3	4	5
(f) Men are reluctant to ask me out	1	2	3	4	5
(g) It is difficult to find shoes that fit	1	2	3	4	5
(h) I can never avoid being noticed	1	2	3	4	5
(i) It has limited my work/career options	1	2	3	4	5

C12 Overall, being tall has increased my opportunities to

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.....☐☐

C13 Overall, being tall has decreased my opportunities to

.....

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.....

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.....☐☐

C14 As far as you can recall, why did your family seek a medical opinion about your height?

[circle as many numbers as appropriate]

(a) Father's concern about his daughter being "too tall"	1
(b) Mother's concern about her daughter being "too tall"	2
(c) I was unhappy	3
(d) Family was told treatment was available	4
(e) Media coverage about availability of treatment	5
(f) Mother's personal experience of tall stature	6
(g) Difficulties at school	7
(h) Difficulty finding clothes that fitted	8
(i) Don't know	9
(j) Other (please specify)	10

Section C continued

The next group of questions ask about assessment and treatment you received as a tall girl.

C15 As far as you can recall, who first suggested that you should have a medical assessment for your height?

[circle one number]

Doctor	1
Family friend/neighbour	2
Parents together	3
Father	4
Mother	5
School teacher	6
Other (please specify)	7

C16 How old were you, when you were first assessed?

☐☐ . ☐☐ years months

C17 When you were assessed as a girl, what height did the doctor predict you would grow to as an adult?

☐ ☐☐ OR ☐☐☐ feet inches cm

☐ Don't know/don't remember

C18 Who made the decision to have or not to have treatment?

[circle only one number]

Father	1
Mother	2
Parents together	3
I did	4
I did with my parents	5
Don't know	6
Other (please specify)	7

C19 Do you think you had an active say in making the decision about whether or not to have treatment to reduce your adult height?

Yes	1
No	2

Comments.....

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.....☐☐

Section C continued

C20 Did you receive treatment to reduce your adult height?

[circle one number]

Yes	1
No	2
Don't know	3

C21 On balance, are you satisfied with the decision that was made about whether or not to have treatment?

[circle one number]

Yes	1
No	2

Comments.....

.....

If you did not receive treatment for tall stature go to C28

If you cannot remember the exact dates for the next two questions please give an estimate.

C22 How old were you when you started treatment? ☐☐ . ☐☐ years months

C23 How old were you when you completed treatment? ☐☐ . ☐☐ years months

C24 What was the name of the drug you took? *[circle as many numbers as applicable]*

DES (diethylstilboestrol or stilboestrol)	1
EE (ethinyl estradiol)	2
Other (please specify)	3
Not sure/cannot remember name	4

C25 If you can remember, when you were prescribed this treatment did you ..... ?

[circle one number]

Take most of the tablets regularly	1
Take most of them irregularly	2
Take the tablets infrequently	3
Cannot remember	4

Comments.....

.....

..... ☐☐

Section C continued

C26 Did you ever experience any of the following side effects whilst on this treatment?

☐ NO side effects experienced *(tick box if applicable)*

[Circle as many numbers from 1-22 as applicable]

Problematic weight gain	1	Ovarian cysts	11
Heavy periods	2	Nausea	12
Irregular periods	3	Vomiting	13
Pigmentation on face	4	Improved skin	14
Increased pigmentation of nipples	5	Excessive sweating	15
Dark line from navel to pubic hair (linea nigra)	6	Thrombosis (blood clot)	16
		Calf cramps	17
Pigmentation elsewhere	7	Depression	18
Increased vaginal secretions	8	Aggression	19
Leaking breasts (galactorrhoea)	9	Mood swings	20
Breast pain	10	Breast lumps	21
Other (please specify)			22

C27 After treatment had finished, did you ever notice a spontaneous leakage of milk or discharge from your breasts?

[circle one number]

Yes	1	If YES, how long did this last? ___ ___ ☐days or ☐weeks
No	2	
Don't know	3	

C28 How do you think your parents felt about the height you achieved when you finished growing?

[Circle one choice for (a) and for (b)]

(a) MOTHER ☐ Not applicable		(b) FATHER ☐ Not applicable	
Very happy	1	Very happy	1
Happy	2	Happy	2
Satisfied	3	Satisfied	3
Mixed feelings	4	Mixed feelings	4
Disappointed	5	Disappointed	5
Other (please specify)	6	Other (please specify)	6
.....		.....	

Section C continued

C29. Women have reported a broad range of experiences of being assessed and treated for tall stature. The next few questions ask about your experience and how you felt about the assessment and treatment procedures.

How often, if ever, did you experience the following when you were assessed or treated for tall stature?

[circle one number on each line]

	Never	1-2 times	3-5 times	6 or more times
Height measured	1	2	3	4
Being weighed	1	2	3	4
Having X-Rays	1	2	3	4
An explanation from the doctor about what assessment involved	1	2	3	4
Breast examination	1	2	3	4
Genital examination	1	2	3	4
Blood tests	1	2	3	4
Being photographed	1	2	3	4
An explanation from the doctor about what treatment involved	1	2	3	4
Other (please specify)	1	2	3	4
.....				

C30 **Was your mother or other close relative present in the room with you for any of the following procedures?**

[circle one number on each line]

	Yes	No	Cannot remember	Not Applicable
Height measured	1	2	3	4
Being weighed	1	2	3	4
Having X-Rays	1	2	3	4
An explanation from the doctor about what assessment involved	1	2	3	4
Breast examination	1	2	3	4
Genital examination	1	2	3	4
Blood tests	1	2	3	4
Being photographed	1	2	3	4
Other (please specify)	1	2	3	4
Comments				
.....				

C30a **Was anyone present during any of these procedures who you did not want to be there?**

Yes 1

No 2 go to C31

Section C continued

C30b **If YES, who was there and for which procedure(s) were they unwelcome?**

Person	Procedure
.....	.....
.....	.....
.....	.....

C31 **Were you undressed for any of these procedures?**

[circle one number on each line]

	Never had this procedure	Not undressed	Undressed to underwear	Fully undressed
Height measured	1	2	3	4
Being weighed	1	2	3	4
Being photographed	1	2	3	4
Other (please specify)	1	2	3	4

C32 **How did you feel when you were having the tests or procedures that are listed below?**

[circle one number on each line]

	Special	Comfortable	Indifferent	Uncomfortable	Embarrassed
Height measured	1	2	3	4	5
Being weighed	1	2	3	4	5
Having X-Rays	1	2	3	4	5
Breast examination	1	2	3	4	5
Genital examination	1	2	3	4	5
Blood tests	1	2	3	4	5
Being photographed	1	2	3	4	5
Other (please specify)	1	2	3	4	5

C33 **In your opinion what were the best aspects of the assessment/treatment?**

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C34 In your opinion what were the worst aspects of the assessment/treatment?

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.....☐☐

C35 Overall, what best describes your experience of the assessment/treatment procedures for tall stature?

[circle as many numbers from 1-13 as applicable]

I felt supported	1	I was distressed	7
It was embarrassing	2	I felt reassured	8
I felt special	3	I felt uncomfortable	9
It was intrusive	4	It was painful	10
It was acceptable	5	I felt indifferent	11
It was scary	6	I was comfortable	12
Other (please specify)			13

C36 If a friend was concerned that their daughter was going to be very tall, what advice would you give?

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.....☐☐

C37 Now we are going to ask about the heights of your family members:

Family heights - please complete the table below. If you are unsure about any measurements give an estimate.

		Not applicable	
C37a	How tall is/was your mother?	☐	☐☐☐ feet ☐☐ in OR ☐☐☐ cm
C37b	How tall is/was your father?	☐	☐☐☐ feet ☐☐ in OR ☐☐☐ cm
C37c	How tall is/was your tallest sister?	☐	☐☐☐ feet ☐☐ in OR ☐☐☐ cm
C37d	How tall is/was your tallest brother?	☐	☐☐☐ feet ☐☐ in OR ☐☐☐ cm

C38 Were any of your sisters or brothers assessed for tall stature?

[circle one number for (a) and (b)]

(a) Sister(s)		(b) Brother(s)	
YES	1	YES	1
NO	2	NO	2
Don't have any sisters	3	Don't have any brothers	3
Don't know	4	Don't know	4

Section C continued

C39 Were any of your sisters or brothers treated for tall stature?

[circle one number for (a) and (b)]

(a) Sister(s)		(b) Brother(s)	
YES	1	YES	1
NO	2	NO	2
Don't have any sisters	3	Don't have any brothers	3
Don't know	4	Don't know	4

Section D These questions ask about your general health and lifestyle

D1 How many times have you consulted the following people for your own health in the last 12 months?

[circle only one number on each line]

	None	1-2 times	3-4 times	5-6 times	7 or more times
Family doctor or another general practitioner	1	2	3	4	5
A hospital doctor (eg in outpatients or casualty)	1	2	3	4	5
A specialist doctor	1	2	3	4	5
An allied health professional (eg optician, dentist, physiotherapist, podiatrist, dietician, counsellor etc)	1	2	3	4	5
An "alternative" health practitioner (eg herbalist, chiropractor, naturopath, acupuncturist, etc)	1	2	3	4	5

D2 Have you ever been told by a doctor that you have:

[circle one number on each line]

	YES	NO
(a) Diabetes (high blood sugar)	1	2
(b) Heart disease	1	2
(c) Hypertension (high blood pressure)	1	2
(d) Stroke	1	2
(e) Thrombosis (blood clot)	1	2

(f) Low iron level (anaemia)	1	2
(g) Asthma	1	2
(h) Bronchitis/emphysema	1	2
(i) Osteoporosis	1	2
(j) Cancer (please specify)	1	2
(k) Other major illness (please specify)	1	2
.....		
.....		

Section D continued

D3 In the last 12 months have you had any of the following:

[circle one number on each line]

	Never	Rarely	Sometimes	Often
(a) Allergies, hay fever, sinusitis	1	2	3	4
(b) Breathing difficulty	1	2	3	4
(c) Indigestion/heartburn	1	2	3	4
(d) Chest pain	1	2	3	4
(e) Headaches/migraines	1	2	3	4
(f) Severe tiredness	1	2	3	4
(g) Stiff or painful joints	1	2	3	4
(h) Back pain	1	2	3	4
(i) A broken bone (fracture)	1	2	3	4
(j) Urine that burns or stings	1	2	3	4
(k) Leaking urine	1	2	3	4
(l) Constipation	1	2	3	4
(m) Haemorrhoids (piles)	1	2	3	4
(n) Other bowel problems	1	2	3	4
(o) Vaginal discharge or irritation	1	2	3	4
(p) Premenstrual tension	1	2	3	4
(q) Irregular monthly periods	1	2	3	4
(r) Heavy periods	1	2	3	4
(s) Severe period pain	1	2	3	4
(t) Hot flushes	1	2	3	4
(u) Night sweats	1	2	3	4
(v) Skin problems	1	2	3	4
(w) Eyesight problems	1	2	3	4
(x) Hearing problems	1	2	3	4
(y) Difficulty sleeping	1	2	3	4

(z) Other (please specify)	1	2	3	4
.....				

Section E: Health and social behaviours

These next few questions ask about screening for breast and cervical cancer.

E1 A breast examination is when the breasts are felt for lumps to detect possible breast cancer.

Have you **ever** had a breast examination by a doctor or other health professional?

Yes	1	
No	2	go to E4

E2 Do you have **regular** breast examinations by a doctor or other health professional?

Yes	1	
No	2	go to E4

E3 How often do you have a breast examination by a doctor or other health professional?

Monthly	1
6 Monthly	2
Yearly	3
Other (please specify.....)	4

E4 Do you **regularly** examine your breast for lumps?

Yes	1	
No	2	go to E6

E5 How often do you examine your breasts?

Monthly	1
6 Monthly	2
Yearly	3
Other (please specify)	4

E6 A mammogram is an X-ray taken of the breast by a machine that presses against the breast while the picture is taken.

Have you **ever** had a mammogram?

Yes	1	
No	2	go to E10

E7 What is the **usual** time period between your mammograms?

Specify number of years	☐☐	1
Only had one		2

Section E continued

E8 When did you have your **last** mammogram?

Less than one year ago	1
1 year to less than 2 years ago	2
2 years to less than 3 years ago	3
3 years to less than 4 years ago	4
4 years to less than 5 years ago	5
5 or more years ago	6

E9 Why did you have this mammogram?

Symptoms present	1
Family history of breast cancer	2
Had breast cancer in the past	3
General check-up	4
Don't know	5
Other (specify)	6

E10 Have you ever been diagnosed as having breast cancer?

Yes	1	
No	2	go to E12

E11 How old were you when you were first diagnosed with breast cancer?

☐☐ YEARS

E12 A Pap Smear Test, sometimes called a Pap Test, is a routine test carried out by a doctor or nurse. It is recommended for all women for early detection of cancer of the cervix.

Have you **ever** had a Pap Smear Test?

Yes	1	
No	2	go to E15

E13 When did you have your last Pap Smear Test?

Less than 1 year ago	1
1 year to less than 2 years ago	2
2 years to less than 3 years ago	3
3 years to less than 4 years ago	4
4 years to less than 5 years ago	5
5 or more years ago	6

E14 What is the **usual** time period between your Pap Smear Tests?

Specify number of years	☐☐	1
Less than one year		2
Only had one		3

Section E continued

E15 **Now we would like to ask you some questions about SMOKING**

Which of the following best describes your smoking status now?

[circle one number]

I have never smoked	1	Go to E21
I used to smoke	2	Go to E16
I now smoke occasionally	3	Go to E18
I now smoke regularly	4	Go to E18

E16 If you used to smoke, how long ago did you give up smoking?

[circle one number]

Within the last 6 months	5
6 - 12 months ago	6
1-5 years ago	1
6-10 years ago	2
11-20 years ago	3
More than 20 years ago	4

E17 If you used to smoke, how many cigarettes did you usually smoke in a day?

[circle one number]

1-19 a day	1
20-39 a day	2
40-59 a day	3
60-79 a day	4
80 or more a day	5

E18 If you now smoke, how many cigarettes do you usually smoke in a day?

[circle one number]

1-19 a day	1
20-39 a day	2
40-59 a day	3
60-79 a day	4
80 or more a day	5

E19 At what age did you start smoking? ☐☐ YEARS

E20 Have you ever smoked daily for six months or more?

[circle one number]

Yes	1
No	2
Never smoked	3

Section E continued

E21 **Now we would like to ask you some questions about DRINKING ALCOHOL**

How often do you usually drink alcohol?

[circle one number]

I never drink alcohol	1	go to F1
I drink rarely	2	
Less than once a week	3	
On 1 or 2 days a week	4	
On 3 or 4 days a week	5	
On 5 or 6 days a week	6	
Every day	7	

A "standard drink" is a small glass of wine or middy of beer, a nip of spirits, or a mixed drink

E22 On a day when you drink alcohol, how many drinks do you usually have?

[circle one number]

1 or 2 drinks per day	1
3 or 4 drinks per day	2
5 - 8 drinks per day	3
9 or more drinks per day	4

E23 How often do you have five or more standard drinks of alcohol on one occasion?

[circle one number]

Never	1
Less than once a month	2
About once a month	3
About once a week	4
More than once a week	5

SECTION F: These questions ask about you and your life

F1 What is your date of birth? ☐☐/☐☐/19☐☐
day month year

F2 What is your present marital status?

[circle one number]

Married	1
De facto (opposite sex)	2
De facto (same sex)	3
Separated	4
Divorced	5
Widowed	6
Single	7

F3 What is the highest level of education you have completed?

[circle one number]

Primary school	1
Intermediate Certificate/Year 10 (or equivalent)	2
Higher School &/or Leaving Certificate/Year 11 & 12 (or equivalent)	3
Trade/apprenticeship (eg Hairdresser, Chef)	4
Certificate/diploma (eg Child Care, Technician)	5
University degree	6
Higher University degree (eg Grad Dip, Masters, PhD)	7

F4 Which of the following best describes your main current employment status?

[circle one number]

In full time paid work	1
In part time or casual paid work	2
Work without pay (eg in a family business)	3
Home duties only - no paid work	4
Studying - no paid work	5
Unemployed - looking for work	6
Unpaid voluntary work	7
Retired	8
Unable to work due to sickness or injury	9
Other (please specify)	10

F5 What is/was your main occupation and if you have a partner, what is their main occupation?

	SELF	PARTNER
[circle one number for yourself and one for partner]		☐ *[Tick box if no partner]*
Manager or Administrator (eg personnel manager, managing supervisor)	1	1
Professional (eg teacher, social worker, doctor, artist)	2	2
Para-professional (eg welfare worker, technical officer, registered nurse, police)	3	3
Trade (eg hairdresser, cook, mechanic)	4	4
Administrative assistant (eg secretary, telephonist)	5	5
Sales and personal service worker (eg sales assistant, bar attendant, child care worker, enrolled nurse)	6	6
Machine operator or driver (eg sewing machinist)	7	7
Manual worker (eg labourer, cleaner, kitchenhand)	8	8
Never had a paid job	9	9
Other (please specify)	10	10

F6 What is your total annual family income before tax (including pensions and dividends)?

[circle the number that applies to you]

$0 - $25,999	1
$26,000 - 51,999	2
$52,000 - 77,999	3
$78,000 - 103,999	4
$104,000 +	5

F7 Do you have anything else you would like us to know?

.....

.....

.....

.....

.....

.....

.....

.....

.....

F8 The results of this study will be available in approximately two years. Would you like us to send you a copy of the results?

Yes 1 (please notify us of any change of address, if known write new address below)

No 2

Tall Girls Study

The next stage of the study involves a telephone interview. If you agree to take part, you will be contacted by an interviewer from Millward Brown. This professional organisation will be conducting the interviews on our behalf. The interviewer will know only your first name and telephone number. They will not know about your assessment or treatment and will not have access to the answers you have provided in this postal questionnaire.

So that we can arrange the telephone interview please

- provide your telephone number(s)
- indicate the times you can be contacted
- complete the top part of yellow consent form, on the back page of this questionnaire, regarding the telephone interview.

Telephone numbers (I) (.....) .....**home/work**

(II) **mobile** .....

Best time(s) to contact you:			
	AM	PM	EVENING
Monday			
Tuesday			
Wednesday			
Thursday			
Friday			
Saturday			
Sunday			

Finally, we would like to contact the medical practitioner who assessed or treated you, so that we can confirm details about your assessment and, if appropriate, treatment for tall stature. To give us permission, please complete the lower part of consent form, on the back page of this questionnaire.

Your contribution has been very valuable.

Thank you very much for the time and trouble you have taken in completing this questionnaire.

Tall Girls Study

FINAL CHECK LIST

1	If you have left your height measurement to do until a time before 12 midday, have you now filled in this height measurement (C1)?
2	Have you signed the consent form for the telephone interview on the adjacent page?
3	If you are willing to provide contact details of your doctor or hospital where you were treated, have you completed and signed the lower portion of consent form?

This questionnaire includes the SF-36™ Health Survey. Item numbers A1 to A11 in this questionnaire.
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Study ID number: _ _ _ _ _

CONSENT FORM

The long-term health and psychosocial effects of oestrogen treatment to reduce the adult height of tall girls.

Chief Investigators:

DR ALISON VENN
Centre for the Study of Mothers' & Children's Health, La Trobe University and Menzies Centre for Population Health Research, University of Tasmania

PROFESSOR JUDITH LUMLEY & DR PRISCILLA PYETT
Centre for the Study of Mothers' & Children's Health, La Trobe University

PROFESSOR GEORGE WERTHER
Centre for Hormone Research, Royal Children's Hospital Research Institute

(A) Consent to participate

I have read and understood the information on the study information sheet. Any questions I have asked have been answered to my satisfaction.

I agree to participate in the project by:

- filling in the questionnaire
- providing information on my current height and weight
- participating in the telephone interview

I realize I may withdraw at any time. I agree that research data provided by me or with my permission during the project may be included in a thesis, presented at conferences and published in journals on the condition that neither my name nor any other identifying information is used.

Name of participant .....

Signature ..... Date ...../...../.....

(B) Consent to obtain medical information

We would like to contact the doctor(s) who assessed and/or treated you for tall stature. We will, however, only do this with your permission. We are requesting access to your medical records so that we can confirm details about your height assessment and, if applicable, your treatment for tall stature. All the information we collect will be kept confidential. The information we collect will be used solely for the purposes of this research study.

Please provide us with the name and address(es) of the doctor or hospital who assessed or treated you for tall stature, and the year that you attended. If you are unsure of a doctor's name or address, please give us whatever information you do have.

(1) Dr or hospital.....Year attended .....

Address .....

.....State..... Postcode.....

(2) Dr or hospital.....Year attended .....

Address .....

.....State..... Postcode.....

Your name ..... (print name) Maiden/other surname(s) used .....

Your signature ..... Date ...../...../.....

APPENDIX 4

Follow-up 1 CATI questionnaire

CONFIDENTIAL

ID no: __ __ __ __ __ __

Date _____

Interviewer .....

Time start:.....

Time finish:.....

The Tall Girls Study

CATI Questionnaire -HARD COPY

Hi I'm ..... form La Trobe University .....

Your answers to this questionnaire are confidential and your participation is voluntary. The questions I am about to ask include details of your

- Menstrual and reproductive history,
- Psychological wellbeing
- and Sexual experiences.

Preliminary research with treated and untreated tall women has suggested that these areas of women's health should be explored.

The questions I will be asking have been used in a number of other studies in Australia and elsewhere and everyone in this study will be asked this same set of questions.
If at anytime you wish me to skip a question or stop this telephone call please let me know.

This call may be monitored for quality assurance purposes, if you do not wish your call to be monitored please let me know.

Now the first group of questions is about your medical history

.....in particular your gynaecological health

SECTION A Medical History – Gynaecological conditions

The first two questions are about whether you have had any gynaecological health problems or procedures

A1 Has a doctor ever diagnosed you as having (CONDITION)- I'll read my list	YES	NO	If YES, In what year did you FIRST have (CONDITION).
Endometriosis?	1	2	☐☐☐☐
An abnormal Pap Smear or Smear Test?	1	2	☐☐☐☐
Cervical dysplasia, CIN, or CIS? [cervical intraepithelial neoplasia or carcinoma in situ]	1	2	☐☐☐☐
A benign tumour of the reproductive organs eg fibroid? if YES, specify	1	2	☐☐☐☐ ☐☐☐☐
Cancer of the reproductive organs If YES, what kind of cancer?	1	2	☐☐☐☐ ☐☐☐☐
Any other cancer? If YES, what kind of cancer?	1	2	☐☐☐☐ ☐☐☐☐

A2 Have you ever had (PROCEDURE)? I'll read my list	YES	NO	If YES, In what year did you FIRST have (PROCEDURE)?
A hysterectomy [removal of uterus +/- ovaries]	1	2	☐☐☐☐
Exploratory pelvic surgery, eg. a laparoscopy [incision into the abdomen to look at reproductive organs]?	1	2	☐☐☐☐
An ovarian cyst removed	1	2	☐☐☐☐
Laser therapy of the cervix, cryosurgery or cautery of the cervix	1	2	☐☐☐☐
Cervical suture or stitch [inserted during pregnancy]	1	2	☐☐☐☐
Endometrial ablation [diathermy or laser of uterine lining]	1	2	☐☐☐☐
Other gynaecologic surgery	1	2	
• If YES to "other gynaecological surgery", • [please specify .....]			☐☐☐☐
A breast biopsy [sample of breast tissue]	1	2	☐☐☐☐
A mastectomy [surgery to remove breast]	1	2	☐☐☐☐
Other breast surgery	1	2	
• If YES to "other breast surgery", • [please specify .....]			☐☐☐☐

A2b(i) Have you ever had a D & C [dilatation and curettage]?

Yes 1 (If Yes), how many have you had? ☐

No 2 go to A3

A2b(ii) If yes, how many of your D& Cs were related to a pregnancy condition? ☐

SECTION B : Reproductive History

These next few questions are about your ability to become pregnant

B1 Have you ever tried to become pregnant for 12 months or more without succeeding?
YES 1
NO 2
DK 3

B2 Have you ever seen a doctor because you were having trouble getting pregnant?
YES 1
NO 2 go to C1

B3 Did you have (PROCEDURE)? I'll read my list	YES	NO	DK	If YES, year first had this (PROCEDURE)
To chart your temperature?	1	2	3	☐☐☐☐
A test of your hormone levels?	1	2	3	☐☐☐☐
A post-coital test of your cervical mucus?	1	2	3	☐☐☐☐
A hysterosalpingogram, an x-ray in which dye is put into the fallopian tubes to look for a blockage?	1	2	3	☐☐☐☐
An endometrial biopsy, a sample of the lining of the uterus?	1	2	3	☐☐☐☐
A laparoscopy [incision into the abdomen to look at reproductive organs]?	1	2	3	☐☐☐☐

B4 Has a doctor ever told you (or your partner) that you have (DIAGNOSIS) I'll read my list	YES	NO	DK
a An ovulatory problem?	1	2	3
b A tubal problem?	1	2 → go to B4c	3 → go to B4c
(b1) If YES, was the problem in one or both tubes?	One tube	Both tubes	3
c A uterine problem?	1	2	3
d A cervical mucus problem?	1	2	3
e A hormonal problem?	1	2	3
f Semen abnormalities?	1	2	3
g Sperm antibodies?	1	2 → go to B4h	3 → go to B4h
(g1) If YES, are the antibodies to your partner's sperm?	1	2	3
(g11) If YES, does your partner have antibodies to his own sperm?	1	2	
h Any other identified fertility problem?	1	2	3
(h1) If YES, what was the problem(s)?			
.....			
.....			
i An unexplained fertility problem	1	2	3

B5a Have you ever taken fertility drugs for the treatment of infertility?
Yes 1
No 2 go to C1

B5b If YES to taking fertility drugs, ☐☐ number of cycles
What is the total number of cycles of fertility drug treatment that you've had? Prompt ☐ 1-2, ☐ 3-5, ☐ 6-11, ☐ >12

SECTION C: Time to first pregnancy

The next couple of questions I am going to ask are about becoming pregnant

C1 Have you ever been pregnant? Including all your pregnancies: miscarriages, stillbirths, terminations, molar or tubal pregnancies, as well as live births or current pregnancy.
Yes, 1 C2
(if YES) how many times have you been pregnant ☐
No 2 go to C19

Now I would like to ask you some questions about pregnancy(ies) starting with your first pregnancy.

	C2. In what MONTH and YEAR did your (#) pregnancy end?	C3. How did your (#) pregnancy end? Circle one number below	C4. How much did this baby weigh at birth? Include MULTIPLE BIRTHS	C5. Was/were this/these baby (babies) born early, late or on time? *Circle number below*	C6. How many weeks (early/late)?	C7. How many weeks did this pregnancy last - from your last normal menstrual period?
Preg 1	☐☐/☐☐☐☐ ☐ currently pregnant go to C13	1 live birth →(C4) 2 stillbirth →(C7) 3 miscarriage/blighted ovum →(C7) 4 induced/elective termination →(C7) 5 tubal/ectopic pregnancy →(C7) 6 molar pregnancy/hydatid mole →(C7)	☐☐☐☐ gm or lbs ☐☐ oz	1 - early 2 - on time →(C7) 3 - late	☐☐ weeks → go to NEXT PREGNANCY or C8	——— Weeks
Preg 2	☐☐/☐☐☐☐ ☐ currently pregnant go to C13	1 live birth →(C4) 2 stillbirth →(C7) 3 miscarriage/blighted ovum →(C7) 4 induced/elective termination →(C7) 5 tubal/ectopic pregnancy →(C7) 6 molar pregnancy/hydatid mole →(C7)	☐☐☐☐ gm or lbs ☐☐ oz	1 - early 2 - on time →(C7) 3 - late	☐☐ weeks → go to NEXT PREGNANCY or C8	——— weeks
Preg 3	☐☐/☐☐☐☐ ☐ currently pregnant go to C13	1 live birth →(C4) 2 stillbirth →(C7) 3 miscarriage/blighted ovum →(C7) 4 induced/elective termination →(C7) 5 tubal/ectopic pregnancy →(C7) 6 molar pregnancy/hydatid mole →(C7)	☐☐☐☐ gm or lbs ☐☐ oz	1 - early 2 - on time →(C7) 3 - late	☐☐ weeks → go to NEXT PREGNANCY or C8	——— weeks
Preg 4-6	☐☐/☐☐☐☐ ☐ currently pregnant go to C13	1 live birth →(C4) 2 stillbirth →(C7) 3 miscarriage/blighted ovum →(C7) 4 induced/elective termination →(C7) 5 tubal/ectopic pregnancy →(C7) 6 molar pregnancy/hydatid mole →(C7)	☐☐☐☐ gm or	1 - early 2 - on time →(C7) 3 - late	☐☐ weeks → go to NEXT PREGNANCY or C8	——— weeks

C1 contd, Now I would like to ask you some questions about BREASTFEEDING, starting with your first child.

	C8. Did you commence breastfeeding this baby?	C9. How long did your baby have breast milk only?	C10. How long, did you breastfeed this baby all together (including when this baby had formula &/or solids)	C11a. Why did you stop breastfeeding this baby? *(Ask as an open ended question and enter numbers that apply)* See table C11b	C12a. If you didn't commence breastfeed this baby -what was the reason? *(Ask as an open ended question and enter numbers that apply)* See table C12b	C13. Did you notice that your breast increased in size while pregnant?
BABY 1	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	ENTER NUMBER(s) ☐☐☐☐ →go to C13	ENTER NUMBER(s) ☐☐☐☐ →go to C	YES ☐ NO ☐
BABY 2	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	ENTER NUMBER(s) ☐☐☐☐ →go to C13	ENTER NUMBER(s) ☐☐☐☐ →go to C	YES ☐ NO ☐
BABY 3	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	ENTER NUMBER(s) ☐☐☐☐ →go to C13	ENTER NUMBER(s) ☐☐☐☐ →go to C	YES ☐ NO ☐
BABY 4	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	ENTER NUMBER(s) ☐☐☐☐ →go to C13	ENTER NUMBER(s) ☐☐☐☐ →go to C	YES ☐ NO ☐
BABY 5	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	ENTER NUMBER(s) ☐☐☐☐ →go to C13	ENTER NUMBER(s) ☐☐☐☐ →go to C	YES ☐ NO ☐
BABY 6	YES ☐→ C9 NO ☐→ C12	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	☐☐ Days ☐☐ Weeks ☐☐ Months ☐ currently b'feeding	ENTER NUMBER(s) ☐☐☐☐ →go to C13	ENTER NUMBER(s) ☐☐☐☐ →go to C	YES ☐ NO ☐

MILLWARD BROWN to set up for 8 pregnancies and for 8 breastfeeding senarios please

TABLE C11b REASON WHY STOPPED BREASTFEEDING (after having started)	
1	Didn't want to breastfeed or didn't want to breastfeed any longer
2	Nipple trauma
3	Nipple pain
4	Felt there was not enough milk
5	Unable to get baby to attach/suck/difficulties attaching the baby to the breast
6	Baby very premature
7	Lack of help/support/supervision with breastfeeding
8	Mastitis
9	Recurrent mastitis
10	Baby had poor weight gain
11	Advice from professional -who (please state eg GP, psychiatrist)
12	Employment reasons
13	Baby lost interest/always looking around/stopping & starting feeding
14	Breasts didn't fill or became engorged in first few days
15	Other (please specify) .....

TABLE C12b REASONS WHY NEVER ATTEMPTED TO BREASTFEED BABY	
1	Did not want to breastfeed
2	Wanted to bottle feed
3	Employment/work reasons
4	Partner preferred me to bottle feed
5	Family preferred me to bottle feed
6	To avoid the discomfort/pain associated with breastfeeding
7	Baby very premature
8	Body image issues
9	Felt uncomfortable feeding in public
10	So others could share the work of feeding / caring for the baby
11	To regain strength after the birth
12	Other (please specify) .....

C14 The following questions ask about your FIRST pregnancy -

Before your first pregnancy, when you and your partner got pregnant, had you been .....

(i)	trying to get pregnant	1	go to C16a
(ii)	trying NOT to get pregnant	2	
(iii)	not concerned either way	3	

C15a Now I want to ask you a few questions about your use of contraception around the time you conceived your first pregnancy - this includes anything that might prevent pregnancy - such as oral contraceptive pill, condoms, diaphragm, withdrawal, safe days by the calendar, IUD's or any other method.

Around the time you became pregnant, with your first pregnancy, were you or your partner using any method of contraception, for some or all of the time?

Yes	1	
No	2	go to C16a
Cannot remember	3	

C15b Was your use of contraception regular and consistent around that time or was it somewhat irregular? (Tick box that applies)

regular and consistent	1	go to C15d
somewhat irregular	2	

C15c if YES, (somewhat irregular), for how many months in a row had you and your partner been using contraception somewhat irregularly before you became pregnant? _____ no of months

C15d Were you taking the oral contraceptive pill or using an IUD at this time?

Yes	1	go to C18
No	2	go to C18

C16a Was taking the pill, the last method of contraception you used before you got pregnant?

Yes	1	
No	2	go to C17

C16b if YES, some couples wait a few months after stopping the pill before letting themselves get pregnant. Did you use something to prevent pregnancy or avoid sex right after stopping the pill?

Yes	1	
No	2	go to C17

if YES- for how many months did you try to prevent pregnancy right after stopping the pill? ☐☐ No. of months

☐ Cannot remember

C17 Some couples get pregnant right away when they have sexual intercourse without doing anything to prevent pregnancy, others take a long time, or need medical treatment.

For your first pregnancy did you become pregnant during the first menstrual cycle of unprotected intercourse?

Yes	1	go to C18
No	2	
if NO, the second month?		
Yes	1	go to C18
No	2	
if NO, the third month?		
Yes	1	go to C18
No	2	
Please estimate the number of months of unprotected intercourse before you became pregnant	☐☐	no. of months

C18 During the month before you got pregnant, how many times a week, on average, did you usually have sexual intercourse?

☐☐ times per week

☐ Cannot remember

C19 I am going to now ask you a couple of questions that you have already answered, so that I can skip to the next group of questions that will apply to you
Are you (or have you)

	YES	NO	DK
Currently pregnant	1 - go to D27	2	3 - go to D27
Currently breastfeeding	1 - go to D27	2	
Currently taking the oral contraceptive pill or any other prescribed hormone [READ HORMONE BOX]?	1 - go to D27	2	3 - go to D27
Had a hysterectomy	1 - go to D27	2	3
Gone through menopause or the change of life	1 - go to D27	2	3 - go to D27

Hormones include oral contraceptive pills, progestins, and oestrogens.
Some are pills like premarin & provera.
Also, some forms are skin patches, like estraderm, or suppositories.

Section D: MENSTRUAL HISTORY

The next few questions relate to your menstrual periods

D1 How old were you when you got your first menstrual period?

AGE _____ years	1	
DK	2	
Never menstruated	3	go to E1

D2 Do you still have periods?

YES	1	go to D4
NO	2	

D3 For what reasons have your periods stopped?

.....

IF "NO PERIODS" SKIP TO D16

D4 Some women keep a record or calendar of their menstrual cycles. Do you keep any kind of record of the dates of your periods? That record could help you answer these questions. May I hold the phone while you get that record?

YES	1	go to D4b
NO *"that's fine"*	2	go to D5

D4b

Record used	1
Record not used	2

D5 On what date did your most recent period start? _____ / _____ / _ _ _ _
day month year

D6 How sure are you, of when you had your last period?
Are you very sure, fairly sure, or not so sure

Very sure	1
Fairly sure	2
Not so sure	3

D7 On average how many days of bleeding or menstrual flow do you have with your period? Count from the time bleeding or spotting starts _ _ number of days until it completely stops.

D8 Approximately, how often do you have cramps or backache, with your menstrual periods?
Would you say:

Never	1	go to D10
Sometimes	2	
Often	3	
Always	4	
DK	5	

D9 When you have menstrual cramps or backache, how would you describe your pain?

Would you describe your pain as mild, moderate or severe?
[Interviewer to read list below]

DEFINITIONS OF "mild, moderate, severe pain"

Mild	*Your daily activities are not usually affected and pain medication is rarely needed*
Moderate	*Your daily activities may be affected, pain medication is often needed and usually relieves your pain*
Severe	*Your daily activities are definitely affected. Pain medication is needed but often does not relieve the pain.*

Mild	1
Moderate	2
Severe	3
Varies	4
DK	5

D10 **The next three questions are about the LENGTH of your MENSTRUAL CYCLES**
On average, how many days are there from the first day of one period to the first day of your next period? ☐☐ days

Prompt [if too irregular to answer]

D11 What is the **shortest** menstrual cycle you've had in the last 12 months? Again, count how many days there are there from the first day of one period to the first day of your next? ☐☐ days

D12 What is the **longest** menstrual cycle you've had in the last 12 months? ☐☐ days

D13 During the last 12 months did you have any times when you had heavy gushing-type bleeding that was too much for your pads or tampons, even when you changed frequently?

Yes	1	
No	2	*go to D14*

D13b If YES, how often did this happen?

Every period	1
Most periods	2
During occasional periods	3
Just once	4
DK	5

D14 During the past 12 months, did you ever go for more than 6 weeks without having a menstrual period? Please do NOT count times when you were pregnant, breastfeeding, or taking the pill.
Yes 1 If YES, please explain why? .....
.....
No 2

D15 Again, during the last 12 months, have you noticed any changes in:

a the amount of bleeding with your periods?	Yes	1	If YES, is it lighter now	1
	No	2	Heavier now OR	2
			Does it vary: sometimes lighter, sometimes heavier	3
b the total number of days of bleeding with your menstrual periods	Yes	1	If YES, are there	
	No	2	Less days of bleeding now	1
			More days of bleeding now OR	2
			Does it vary: sometimes fewer days, sometimes more	3
c the length of your cycle, that is the number of days from the first day of one period to the first day of the next	Yes	1	If YES, is it shorter now	1
	No	2	Longer now OR	2
			Does it vary: sometimes shorter, sometimes longer	3
d the amount of cramping with your periods	Yes	1	If YES, is the amount less now	1
	No	2	More now OR	2
			Does it vary, sometimes less, sometimes more	3

D16

	Have you ever consulted a doctor about the following (CONDITIONS)? *[I'll read my list]*		D17 If YES, in what YEAR did you first seek medical help for this	D18 Have you ever taken prescribed medication for this?	D19 Was the prescribed medication a hormone? (if YES read out HORMONE BOX below). If YES, go to D20	D20 Are you now taking that hormone for this problem?
a	cramps or backache associated with your periods	YES 1 NO 2 →D16b	☐☐☐☐	YES 1 NO 2	YES 1 NO 2 →D16b DK 3 →D16b	YES 1 NO 2
b	irregular menstrual cycles	YES 1 NO 2 →D16c	☐☐☐☐	YES 1 NO 2	YES 1 NO 2 →D16c DK 3→D16c	YES 1 NO 2
c	PMS (pre-menstrual syndrome)	YES 1 NO 2 →D16d	☐☐☐☐	YES 1 NO 2	YES 1 NO 2 →D16d DK 3 →D16d	YES 1 NO 2
d	heavy or prolonged menstrual bleeding	YES 1 NO 2 →D17e	☐☐☐☐	YES 1 NO 2	YES 1 NO 2 →D16e DK 3 →D16e	YES 1 NO 2
e	absence of a period for at least 6 weeks, not due to pregnancy, breastfeeding or taking the pill *[probe when given a "yes" response]*	YES 1 NO 2 →D16f	☐☐☐☐	YES 1 NO 2	YES 1 NO 2 →D16f DK 3 →D16f	YES 1 NO 2
f	menopause	YES 1 NO 2 →D16g	☐☐☐☐	YES 1 NO 2	YES 1 NO 2 →D16gi DK 3 →D16gi	YES 1 NO 2
gi	other menstrual problem	YES 1 NO 2 →D21				
gii	(please specify)		☐☐☐☐	YES 1 NO 2	YES 1 NO 2 →D21 DK 3 →D21	YES 1 NO 2

(HORMONE BOX) Hormones include oral contraceptive pills, progestins, and oestrogens. Some are pills like premarin and provera. Also, some forms are skin patches, like estraderm, or suppositories.

D21 Have you ever taken the oral contraceptive pill for any reason? YES 1 NO 2 go to D23

D22 For how many years, altogether, have you taken the pill? -not counting the times you may have stopped. YEARS ☐☐ MONTHS ☐☐

D23 Have you ever had night sweats not due to illness?
YES 1
NO 2

D24 Have you ever had hot flushes?
YES 1
NO 2

No sweats and hot flushes - GO TO E1

D25 At what age did you start to have either hot flushes or night sweats?
AGE _____ years 1
DK 2

D26 Have you had either hot flushes or night sweats in the last 3 months?

Yes	1	go to E1
No	2	go to E1

D27 How old were you when you got your first menstrual period?

AGE _____ years	1	*continue*
DK	2	*continue*
Never menstruated	3	*go to E1*

D28 On what date did your most recent menstrual period start? *(if had hysterectomy ask when LAST menstrual period was, can give guesstimate)*

☐☐ /☐☐ /☐☐☐☐

day month year

D29 How sure are you, of when you had your last period? Are you:

Very sure	1
Fairly sure	2
Not so sure	3

D30

	Have you ever consulted a doctor about the following (CONDITIONS)? *[I'll read my list]*		D31 If YES, in what YEAR did you first seek medical help for this	D32 Have you ever taken prescribed medication for this?	D33 Was the prescribed medication a hormone? (if YES read out HORMONE BOX. If YES, go to D34	D34 Are you now taking that hormone for this problem?
a	cramps or backache associated with your periods	YES 1 NO 2 →D30b	☐☐☐☐	YES 1 NO 2 →D30b	YES 1 NO 2 → D30b DK 3 → D30b	YES 1 NO 2
b	irregular menstrual cycles	YES 1 NO 2 →D30c	☐☐☐☐	YES 1 NO 2 →D30c	YES 1 NO 2 → D30c DK 3 → D30c	YES 1 NO 2
c	PMS (pre-menstrual syndrome)	YES 1 NO 2 →D30d	☐☐☐☐	YES 1 NO 2 →D30d	YES 1 NO 2 → D30d DK 3 → D30d	YES 1 NO 2
d	heavy or prolonged menstrual bleeding	YES 1 NO 2 →D30e	☐☐☐☐	YES 1 NO 2 →D30e	YES 1 NO 2 → D30e DK 3 → D30e	YES 1 NO 2
e	absence of a period for at least 6 weeks, not due to pregnancy, breastfeeding or taking the pill *(probe a yes response)*	YES 1 NO 2 →D30f	☐☐☐☐	YES 1 NO 2 →D30f	YES 1 NO 2 → D30f DK 3 → D30f	YES 1 NO 2
f	menopause	YES 1 NO 2 →D30g	☐☐☐☐	YES 1 NO 2 →D30g	YES 1 NO 2 → D30g DK 3 → D30g	YES 1 NO 2
g	other menstrual problem	YES 1 NO 2 →D35				
gi	(please specify)		☐☐☐☐	YES 1 NO 2 →D35	YES 1 NO 2 → D35 DK 3 → D35	YES 1 NO 2

D35 Have you ever taken the oral contraceptive pill for any reason? YES 1 NO 2 go to D37

D36 For how many years, altogether, have you taken the pill? -not counting the times you may have stopped. ☐☐ months ☐☐ years

D37 Have you ever had night sweats not due to illness?

YES	1
NO	2

D38 Have you ever had hot flushes?

YES	1
NO	2

No sweats and hot flushes - go to E1 (CIDI)

D39 At what age did you start to have either hot flushes or night sweats?

AGE _____ years	1
DK	2

D40 Have you had either hot flushes or night sweats in the last 3 months?

Yes	1
No	2

ASK CIDI NOW (sections E, F, G) saying this introduction

"The next group of questions cover some areas that may be personal or sensitive, and you are under no obligation to answer them. Please keep in mind that your answers are treated confidentially and your openness would be gratefully appreciated.'

The questions ask about depression and eating problems and they come from standard questions used in psychological health and well being surveys."

→ Thank you ..... Now we come to the last group of questions.

SECTION H Sexuality and sexual health

These questions ask about your sexuality and sexual health, that is your teenage and adult sex life. They are all questions used in standard surveys about sexual issues. If you feel uncomfortable about any of these questions, you do not have to answer that question. It is important for the validity of the study that women provide honest answers, so we would really appreciate it if you can answer as many as possible.

H1 **Do you currently have a regular sexual partner? Someone you have an ongoing sexual relationship with?**

Yes	1	
No	2	go to H8
Refuse to answer	3	

H2 **Do you live with your regular partner?**

Yes	1
No	2
Refuse to answer	3

H3 **How long have you been in this relationship? (including any time before you were living together)**

Less than 1 year.	1
More than 1 year but less than 2 years.	2
More than 2 years but less than 5 years.	3
More than 5 years but less than 10 years.	4
More than 10 years but less than 20 years.	5
More than 20 years.	6

[INTERVIEWER: if X3 (H1-H3) refuses to answer, go to H8]

H4 **How many times in the past 4 weeks have you had sex with your partner? Even if this wasn't typical for you. Not just intercourse but including other forms of sex.**

☐☐ times

Can't remember.	A
Refuse to answer	B

H5 **Thinking now about your relationship with your partner. How physically pleasurable do you find sex with your partner to be? [ie. within the last 12 months] Is it…**

Extremely pleasurable.	1
Very pleasurable.	2
Moderately pleasurable.	3
Slightly pleasurable.	4
Not pleasurable at all.	5
Refuse to answer	6

H6 **How emotionally satisfying do you find your relationship with your partner to be? [ie. within the last 12 months] Is it…**

Extremely satisfying	1
Very satisfying	2
Moderately satisfying	3
Slightly satisfying	4
Not at all satisfying	5
Refuse to answer	6

H7 **Is your partner?**

Male	1
Female	2
Refuse to answer	3

H8 **How old were you when you first had sex ie, sexual intercourse? [if necessary, clarify this means vaginal intercourse]**

☐☐ years

Can't remember (PROMPT HERE-see list below)	A	
Refuse to answer	B	*[after 3 continuous refuses to answer H16]*
Never had intercourse	C	go to H16

(Any other information/comments offered by respondent)

PROMPT: If - Can't remember or unsure response "As close as you can remember?"

If still unsure PROMPT - "Were you under 18?, 17?, 16?, 15?, 14?.

If still unsure PROMPT - "Do you remember if you were at school? had left school?"

H9a **In your whole life, how many men have you had sex with (ie, sexual intercourse)?**

0	A	go to H12a
☐☐ number of men		
Can't remember [PROMPT HERE-see list below]	C	
Refuses to answer	D	

H9b *PROMPT: "Would that be"?*

☐ *1*	*(If <6 men ask respondent to be exact and write number in box above)*	☐ *6-10*	*(>6 men do NOT need to be exact)*
☐ *2*		☐ *11-20*	
☐ *3-5*		☐ *>20*	

H10a **In the last 5 years [that is, since month year], how many men have you had sex**

with (ie, sexual intercourse)?

0 A go to H12a
☐☐ number of men
Can't remember (PROMPT HERE) C
Refuses to answer D

H10b *PROMPT: "Would that be"?*
☐ *1* *(If <6 men ask respondent to be exact and write number in box above)*
☐ *2*
☐ *6-10* ☐ *11-20* *(>6 men do NOT need to be exact)*

H11a In the last 12 months [that is, since month year], how many men have you had sex (ie, sexual intercourse) with?

0 A go to H12a
☐☐ number
Can't remember (PROMPT HERE) C
Refuses to answer D

H11b *PROMPT: "Would that be"?*
☐ *1* *(If <6 men ask respondent to be exact and write number in box above)*
☐ *2*
☐ *6-10* ☐ *11-20* *(>6 men do NOT need to be exact)*

H12a In your whole life, how many women have you had sex with? [that is, oral sex, or other forms of genital contact]

0 A go to H15
☐☐ number
Can't remember (PROMPT HERE) C
Refuses to answer D

H12b *PROMPT: "Would that be"?*
☐ *1* *(If <6 women ask respondent to be exact and write number in box above)*
☐ *2*
☐ *6-10* ☐ *11-20* *(>6 women do NOT need to be exact)*

H13a In the last 5 years [that is, since month year], how many women have you had sex with?

0 A go to H15
☐☐ number
Can't remember (PROMPT HERE) C
Refuses to answer D

H13b *PROMPT: "Would that be"?*
☐ *1* *(If <6 women ask respondent to be exact and write number in box above)*
☐ *2*
☐ *6-10* ☐ *11-20* *(>6 women do NOT need to be exact)*

H14a In the last 12 months [that is, since month year], how many women have you had sex with?

0 A go to H15

☐☐ number
Can't remember (PROMPT HERE) C
Refuses to answer D

H14b *PROMPT: "Would that be"?*
☐ *1* *(If <6 women ask respondent to be exact and write number in box above)*
☐ *2*
☐ *6-10* ☐ *11-20* *(>6 women do NOT need to be exact)*

[Interviewer: next question: Do not ask women who have NEVER had sexual intercourse]

H15 During sex do you worry whether your body looks unattractive?

Yes 1
No 2
Refuse to answer 3
Comment: .....

Interviewer- Please record any comments, eg Well, Sometimes, Don't think about it, Depends who with etc

[Interviewer: ask everyone]

H16 Do you think of yourself as *(Read out the categories 1-3- if other response then click on 4-6)*

Heterosexual/straight (normal)	1	Queer	4
Lesbian (or gay)	2	Not sure; undecided	5
Bisexual	3	Something else	6
		Other (specify)	7
		Refuse to answer	8

We have finished the main questionnnaire now and
THANK YOU for answering all those questions.

There are just a COUPLE OF GENERAL QUESTIONS to finish with .....

H17 Do you have anything else that you would like us to know?

H18 The results of this study will be available in approximately two years.
Would you like us to send you a copy of the results?

YES (please notify us of any change of address) 1
NO 2

We may do more research in this area and would like to know if you would be happy to be contacted again

YES ☐
NO ☐
Think about it ☐

Thank you very much for your help with this study.

INTERVIEWER'S COMMENTS (-from notes taken during interview)

APPENDIX 5

Literature review of studies that examined the influence of tamoxifen and GnHRA use on mammographic density

Appendix 5: Literature review of studies that examined the influence of tamoxifen and GnHRA use on mammographic density.

Tamoxifen and mammographic density

A review of the literature identified one cross-sectional and nine longitudinal studies that had examined the association between tamoxifen use and mammographic density. These are presented in **Table A5.1**.

Ursin et al. (1996)[1] appeared to be the first to hypothesize and test an association between mammographic density and tamoxifen use. Their preliminary study of 19 women, compared mammographic density before and 12 months after breast cancer treatment with tamoxifen, across three treatment arms: radiation only, tamoxifen with or without radiation, and chemotherapy with our without radiation. Change in density was assessed by a crude subjective five point scale and tamoxifen had a more favourable score, demonstrating a greater reduction in density, compared with the other treatment groups (p=0.03).

Of the eight longitudinal studies since published, all observed a reduction in mean mammographic density, Wolfe grade, or other qualitative scaling system, in tamoxifen users. Those that reported change in percent density included Descenci et al. (2007)[2] , Brisson et al. (2000)[3], Cuzick et al. (2004)[4] (Boyd scale) and Chow et al. (2000)[5]. Reductions of 15.4%, 5.8%, and 6.4% in mean density were observed in tamoxifen users compared with non-users over a period of 1-4.5 years, for the first three of these studies, respectively. Chow et al. (2000)[5] reported a 4.3% mean density reduction with tamoxifen use per year over a period of 2.5 years.

In contrast to the findings of the longitudinal studies above, a cross-sectional study by Tiersten et al. (2004)[6] failed to find an association between tamoxifen use and mammographic density in BI_RADS categories or % density. This study had a small sample size, and study participants were selected regardless of stage or age at time of breast cancer diagnosis or treatment. This is reflected in the large difference in ages between the tamoxifen and control groups (**Table A5.1**). The effect of tamoxifen on mammographic density might be modified by age or stage of disease or treatment. Cuzick et al. (2004)[4] found age to modify the effect of tamoxifen on mammographic density. In women 45 years or younger, tamoxifen appeared to reduce mammographic density by 13.4% (95% CI: 8.6 to 18.1) in contrast to a reduction of 1.1% (95% CI: 3.0 to 5.1) in women 55 years of age or older[4].

Appendix 5: Literature review of studies that examined the influence of tamoxifen and GnHRA use on mammographic density.

As for HRT, it appears that the effect of tamoxifen on mammographic density lasts for only as long as treatment is continued. Slanetz et al.(2004) [7] reported a case-study of a premenopausal woman whose mammographic density returned to normal after she stopped taking tamoxifen. Konez, Goyal and Reavan[8] similarly observed a reversal of effect in 13 of 16 (48%) of women whose density previously reduced with tamoxifen use.

Gonadotropin releasing hormone agonist (GnRHA)

Three studies associated to the same investigative team have examined the effect of GnRHA on mammographic densities and these are described below.

In a US study (1994)[9], 21 premenopausal women with a high risk of breast cancer were randomly assigned to GnRHa (n=14) treatment or a control group (n=7) and followed up after a period of 12 months. The GnRHA treated women were also provided with 'add-back' estrogen and a cyclic progestagen. These steroids were taken in low doses to reduce exposure to the breast while benefiting the cardiovascular and endometrial system. Compared to baseline, GnRHa treated women who had adhered to treatment had a greater reduction in mammographic density (measured visually) compared with untreated women (p =.04). (See **Figure A5.1** for three examples of breast density reduction).

Gram et al. (2001)[10], described a further follow-up of the previous study's cohort. This follow-up measured % density using a more robust computer thresholding technique described in Chapter 6. Women were followed for a period of 24 months while on treatment, and 6-12 months after completion of treatment. Consecutive reductions in percent density from baseline were observed; 9.7% (+/-3.5%; p=0.01) and 11.4% (+/-3.5%, P=0.01) after 12 months and 24 months, respectively. No statistically significant change was observed in the control group –3.2% (+/-3.0%) (p=0.30), –2.5% (+/-2.5) (p=0.47), at 12 months and 24 months, respectively. The reduction in densities was not sustained after cessation of treatment. Six to twelve months after the completion of treatment, mean percent mammographic density returned to baseline levels (See **Figure A5.2**).

Table A5.1: Studies that have examined the association between tamoxifen use and mammographic density.

Study	Country	Study type	Method of Measurement	N	Age (Mean)	Results
Meggiorini et al. (2008) [11]	Italy	Longitudinal (12 months)	BI-RADs	Controls (80) Tamoxifen (68)	63.9 58.5	More women reduced BI-RADs category in tamoxifen group compared with non-tamoxifen group (p=0.021)
Decensi et al. (2007) [2]	Italy	Longitudinal-RCT (12 months)	% density	Placebo and Tamoxifen 1 mg/day, 5mg/day or10mg per week	54	Tamoxifen, particularly in the larger dose of 5 mg/day, induced a larger reduction in mean mammographic density compared to placebo
Tiersten et al. (2004) [6]	US	Cross-sectional	BI-RADS % density categories	Controls (never used) (29) Tamoxifen (ever used) (13)	51 64	Density did not correlate significantly with ever, never, current, or duration of tamoxifen use.(No data presented)
Cuzick et al. (2004) [4]	UK	Longitudinal (54 months)	Boyd Category*	Placebo (430) Tamoxifen (388)		- 7.3% change from baseline (95% CI: 6.1,8.4) p<0.001 - 13.7% change from baseline (95% CI:12.3,15.1) p<0.001‡
Konez, Goyal and Reaven (2001)[8]	US	Longitudinal (2-3, 5 years and 1 year following discontinuation of treatment)	Wolfe grade densiometer	Tamoxifen (24)	67	21% experienced reduction in Wolfe grade 60% experienced reduction based on densiometer.

Study	Country	Study type	Method of Measurement	N	Age (Mean)	Results
Chow et al. (2000)[5]	US	Longitudinal (up to 2.5 yrs)	% density**	Control (20)	51	+/- 0% per yr p= 0.88
				Tamoxifen (32)	50	- 4.3% per yr p=0.0007
Brisson et al. (2000)[3]	Canada	Longitudinal (3.4 years)	Wolfe grade % density	Placebo (33)	50	-3.6% mean percent density 15.2% experienced reduction in Wolfe grade
				Tamoxifen (36)	51	-9.4% mean percent density (p=0.01 vs placebo) 44.4% experienced reduction in Wolfe grade
Atkinson et al. (1999)[12]	UK	Longitudinal (14 months)	Wolfe grade	Control (188)	59	+ 0.03 change in Wolfe grade score (mean)† (p=0.06 vs baseline)
				Tamoxifen (94)		- 0.37 in Wolfe grade score (p=0.001 vs baseline)
Son and Oh (1999)[13]	Korea	Longitudinal (22 months)	Horizontal axis multiplied by vertical axis	Control (70)		28.6% had category decrease
				Tamoxifen (102)		59.8% had category decrease
Ursin et al.(1996)[1]	US	Longitudinal (12 months)	Subjective scale	Radiotherapy	44	0.10 score
				Tamoxifen and radiotherapy	39	0.38 score
				Chemotherapy and radiotherapy (n=19 in total)	40	0.13 score

* Boyd Categories: 0%, 1%–10%, 11%–25%, 26%–50%, 51%–75%, 76%–100%.

** Also used Wolfe Grade and BI-RADS which analysed as continuous category scores

† Mean of scores assigned to each Wolfe Grade: 1 for the most lucent (N1) pattern, 2 for P1, 3 for P2, and 4 for the most dense (DY) pattern.

‡ Regression coefficients for tamoxifen treatment did not differ between univariable and multivariable models – including age and BMI.

Appendix 5: Literature review of studies that examined the influence of tamoxifen and GnHRA use on mammographic density.

Figure A5.1: Mammograms at baseline (left) and 12 months on GnRHA (right) for three participants in the study by Spicer et al. [9]

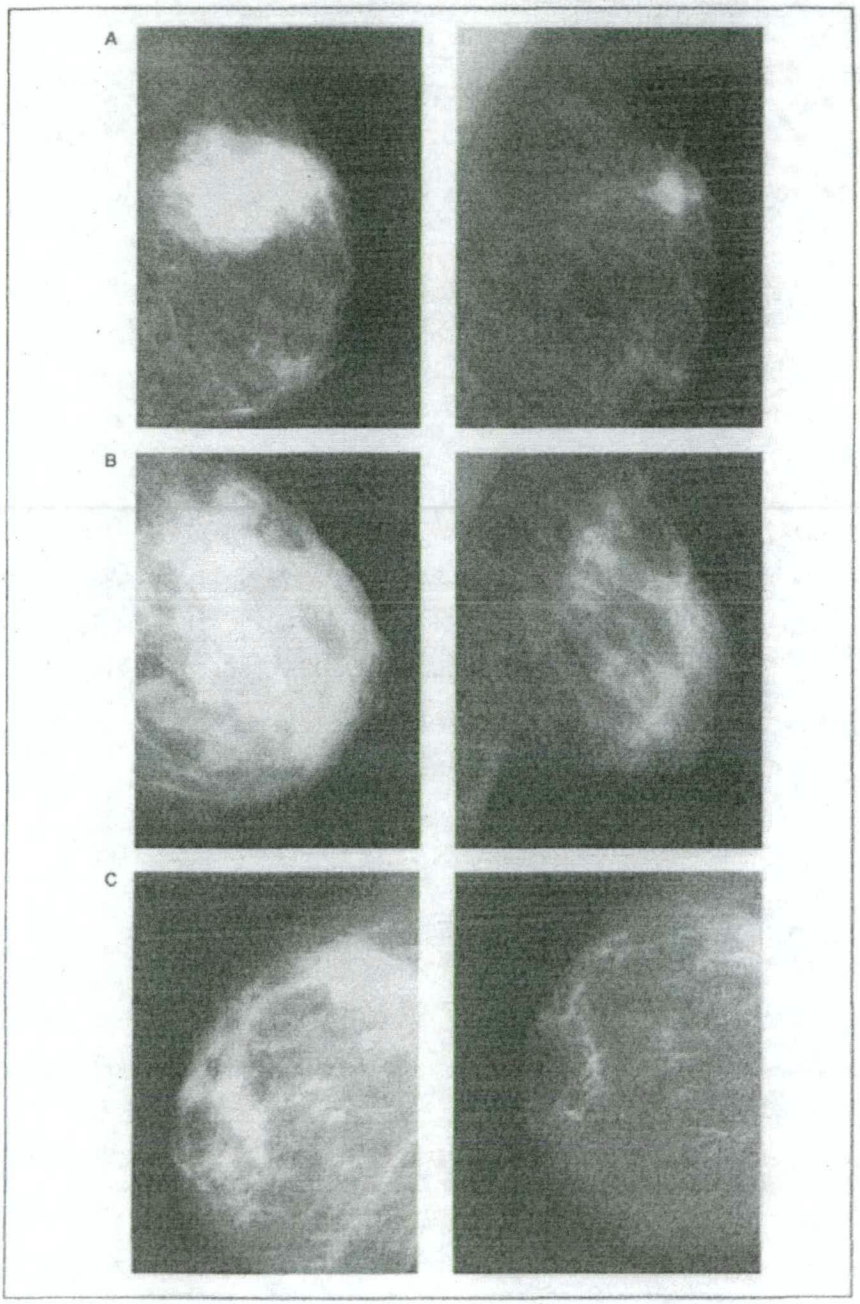
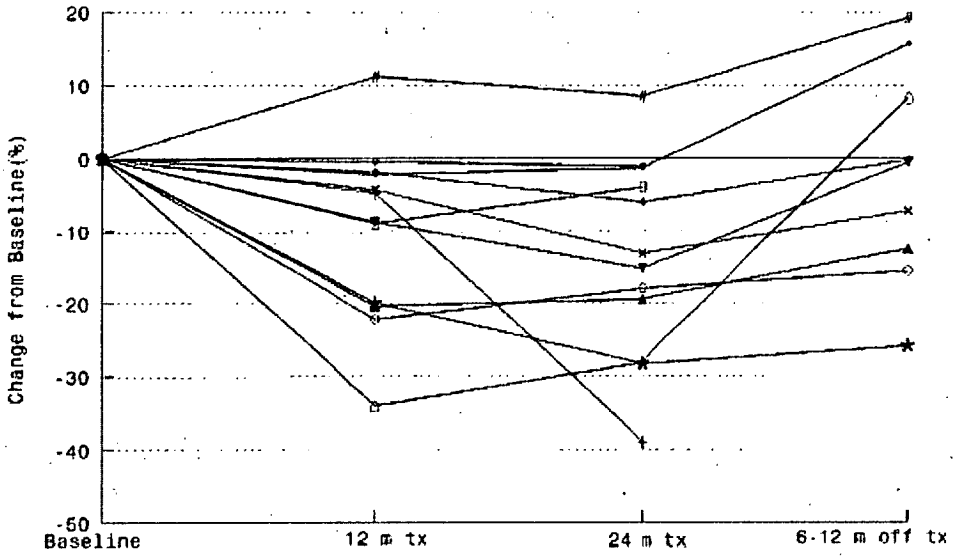


Figure A5.2 Changes in the percent mammographic density from baseline in 13 women treated with GnRHA. Sourced from Gram et al. [10]



The same investigative team undertook a third study on the association between GnRHA and mammographic density in women with a high risk of breast cancer. To be eligible in the previous studies, women either had to have a first degree relative with bilateral breast cancer before 50 years of age, or have had a prior personal diagnosis of lobular carcinoma in situ. In the later study (2007), women had to have had a deleterious BRCA1 mutation. In this study of only 6 women, the median 12 month reduction in percent mammographic density, measured using a computer thresholding technique, was 8.3%; $p = 0.04$. The authors suggested that breast cancer risk could be reduced in BRCA1mut carriers by the GnHRA regimen.

While the treatment regimen involved 'adding back' estrogen and a cyclic progestagen, the overall effect of treatment was to reduce the serum levels of estrogen below normal levels (Spicer et al. 1994)[9]. From the evidence available this reduction appears to result in a corresponding reduction in mean percent density during treatment with GnRHA.

Overall, the evidence presented above suggests that the 'anti-estrogens' tamoxifen and GnHRA reduces mammographic density at least for the duration of treatment.

Appendix 5: Literature review of studies that examined the influence of tamoxifen and GnHRA use on mammographic density.

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Appendix 5: Literature review of studies that examined the influence of tamoxifen and GnHRA use on mammographic density.

APPENDIX 6

Literature review of studies that examined the association between endogenous estrogen and mammographic density

Appendix 6: Literature review of studies that examined the association between endogenous estrogen levels and mammographic density.

Circulating estrogens and mammographic density in postmenopausal women

A search of the research literature identified eight studies that had examined the association between circulating estrogen levels and mammographic density in postmenopausal women. These are summarised in **Table A6.1.** The three forms of circulating estrogen examined in these studies included plasma estradiol (free form and bound) and estrone. One study by Warren et al. (2006)[1] also looked at estrone sulfate, a derivative of estrone. As can be seen in **Table A6.1** three studies[1-3] found no association between each of the estrogens measured and mammographic density. One study[4] observed a statistically significant negative association between percent mammographic density and each of the three estrogen types measured but only in women who had used HRT previously. No association was observed in women who had never used HRT (data not in table). Two studies observed a statistically significant positive association[5,6] between each of the estrogens measured and mammographic density, while two studies observed an association with only one form of estrogen, but not other forms[7,8]. Of these, Boyd et al. (2002)[7] observed a negative association between mammographic density and free estradiol, but not n with bound estradiol, while Bremnes et al. (2007)[8] observed no association between mammographic density and either form of estradiol, nor estrone but did observe a positive association with estrone, but only if IGF-I levels were below the median value for the cohort.

These studies differ across a number parameters including the time period between mammographic density measurement and the taking of blood samples for hormone measurement (e.g. zero difference in the studies by Verheus et al. (2007)[3], Bremnes et al. (2007)[8] and Greendale et al. (2005)[6] and a median/mean difference of eight months in studies by Tamimi et al. (2005)[2] and Boyd et al. (2002)[7], cohort mean estrogen levels and % mammographic density, and sample size (**Table A6.1**). There appears, however, no pattern between the observations of the studies and these differences.

All studies had assessed the effect of age and BMI/body fat/weight on the association between estrogen and mammographic density. Tamimi et al. (2005)[2] and Verheus et al. (2007)[3] observed a negative association between each of the measured estrogens and

mammographic density but the association diminished once the investigators adjusted for BMI/weight height ratio. In contrast, Greendale et al. (2005)[6] initially observed no association between plasma estrogens and percent mammographic density until they adjusted for BMI, after which a positive association was observed. BMI and estradiol levels have been reported to be highly correlated[9]. According to the Melbourne Collaborative Cohort study[10], total and free estradiol has been shown to be highly correlated with BMI but only after six years post-menopause.

BMI is negatively associated with percent density[11]. It is believed that this association is largely due to the dependence of percent density on non-dense area (fatty area of the breast). It is therefore important that BMI is considered in any association concerning percent density. Yet, adjusting for BMI might mask the influence of estradiol levels because of the correlation between estradiol levels and BMI.

Dense area might be a more suitable measure because dense area is not as strongly associated with BMI[12]. While four studies examined the association between dense area and endogenous estrogen levels, all, except the study by Verheus et al. (2007)[3] presented BMI adjusted coefficients. The effect that adjusting for BMI had on any association between mammographic density and endogenous estrogen levels was not detailed in these reports. However, the study by Verheus et al. (2007)[3] suggests that BMI is not masking an association between mammographic density and estrogen levels. This study observed no association despite the lack of BMI adjustment.

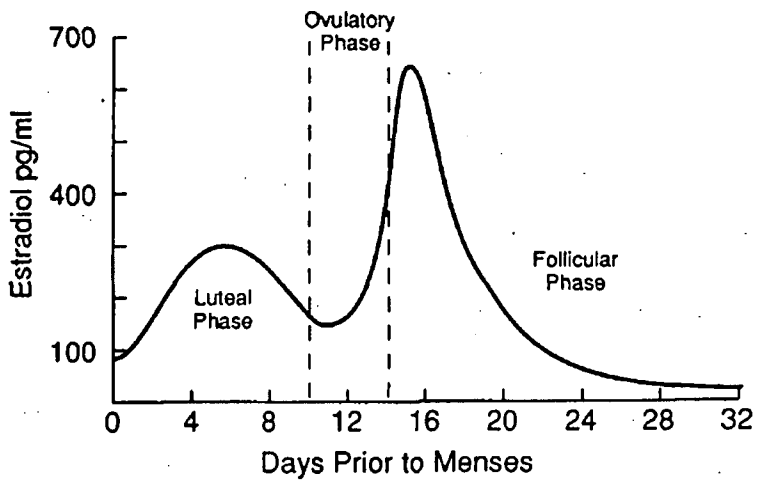
Circulating estrogens and mammographic density in pre-menopausal women.

Three studies examined the association between plasma levels of estrogen and mammographic density in pre-menopausal women (**Table A6.2**). None of these studies found an association between mammographic density and each of the estrogens measured including the study by Meyer et al.(1985) [13] which examined urine levels of estradiol, estrone and estriol.

Postmenopausal women don't encounter the cyclic fluctuations in hormone levels observed in premenopausal women, therefore, the timing of hormone measurement in these studies should not be an issue (**see Figure A6.1**). In the studies on pre-menopausal women, all hormone measurements were taken during the luteal phase of the menstrual cycle.

Appendix 6: Literature review of studies that examined the association between endogenous estrogen levels and mammographic density.

Figure A6.1: Levels of plasma estradiol across the stages of the menstrual cycle.



Sourced from Rosenberg et al. (1994)[14]

Table A6.1: Cross-sectional studies of association between circulating estrogen and mammographic density in postmenopausal women.

Study	Country	N	Density Measure	Hormone	Association	P=value	Conditions/Adjustments
Aiello et al. (2005)[4]	US	88	% density	Estrone	-	0.01	Association in former HRT users only
				Estradiol	-	0.003	
				Free estradiol	-	0.004	
			Dense area	Estrone	-	NS	Adjusted: age, body fat, years since menopause, ethnicity
				Estradiol	-		
				Free estradiol	-		
Tamimi et al. (2005)[2]	US	520	% density	Estrone	0	0.55	Adjusted: age, BMI
				Estradiol	0	0.81	
				Free estradiol	0	0.88	
			Dense area	Estrone	0	NS	
				Estradiol			
				Free estradiol			
Boyd et al. (2002)[7]	Canada	189	% density	Estradiol	0	0.63	Adjusted: age, waist, and sex hormone binding globulin (SHBG)
				Free estradiol	-	0.03	
			Dense area	Estradiol	0	0.76	
				Free estradiol	0	0.06	
Greendale et al. (2005)[6]	US	404	% density	Estrone	+	0.014	Adjusted: age, BMI, parity, prior use of HRT, time since last HRT use, interaction between latter two
				Estradiol	+	0.009	
				Free estradiol	+	0.018	

Study	Country	N	Density Measure	Hormone	Association	P=value	Conditions/Adjustments
Verheus et al. (2007)[3]	Netherlands	775	% density	Estrone	0	0.12	Adjusted: BMI (other variables including age were added to the model but had no effect on the association)
				Estradiol	0	0.58	
				Free estradiol	0	0.34	
			Dense area	Estrone	0	NS	
				Estradiol	0		
				Free estradiol	0		
Bremnes et al. (2007)[8]	Norway	772	% density	Estrone	0	p-trend 0.07	Adjusted: age, BMI, number of children, age at menopause, and HT use
				Estradiol	0	p-trend 0.42	
				Free estradiol	0	p-trend 0.69	
Johansson et al. (2008)[5]	Italy	226	% density	Estrone	+ve	0.02	If IGF-I levels below median
				Estradiol	+ve	0.04	Adjusted: age, BMI
Warren et al (2006)[1]	UK	1,413	% six category	Estrone	0	0.93	Adjusted: BMI, parity, cigarette smoking, years since menopause, and age at menarche Age was significantly associated with density, but not included in the models because it was collinear with years since menopause, which was felt to be the more biologically relevant measure
				Estrone sulfate	0	0.98	
				Estradiol	0	0.91	

Table A6.2: Cross-sectional studies of association between circulating estrogen and mammographic density in premenopausal women.

Study	Country	N	Density Measure	Hormone	Association	P-value	Adjusted
Noh et al. (2006)[15]	US	204	% density	Estrone	0	0.29	Adjusted: age, weight, height, ethnicity, age at menarche, parity, age at first livebirth
				Estradiol	0	0.17	
				Free Estradiol	0	0.45	
			Dense area	Estrone	0	0.77	
				Estradiol	0	0.42	
				Free Estradiol	0	0.91	
Boyd et al. (2002)[7]	Canada	1931	% density	Estradiol	0	0.36	Adjusted: age, waist, and sex hormone binding globulin (SHBG)
				Free estradiol	0	0.40	
			Dense area	Estradiol	0	0.47	
				Free estradiol	0	0.85	
Meyer et al. (1986)[13]	US	110	Wolfe grade	Estrone (urine)	0	NS	-
				Estradiol (urine & plasma)	0		
				Estriol (urine)	0		

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APPENDIX 7

Evidence of an association between IGF-I and mammographic density

IGF-I and Mammographic Density

A number of epidemiological studies have examined the association between IGF-I and mammographic density. The findings of these studies are relevant to this PhD study because girls treated with high-dose estrogens for tall stature have been shown to have reduced circulating IGF-I levels throughout the duration of treatment.

A search of the research literature identified one prospective and ten cross-sectional studies. The cross-sectional studies are summarised in **Table A7.1**. Nine of these examined the association in postmenopausal women. Of these, only one[1] observed a positive association between IGF-I and mammographic density. Of the seven that studied the association in premenopausal women, four, including the large study by Diorio et al. (2005)[2] observed a positive association for both percent density[2] and dense area[3]. Two of the three studies that did not observe a statistically significant association had sample sizes below 250 and according to Diorio and colleagues[2], were not large enough to provide the power to detect a significant association of the size demonstrated in their study for percent mammographic density (correlation coefficient r=0.08, p= 0.02). The third of these studies, by Maskarinec et al. (2007)[4] involved a pooling of four different ethnic cohorts from different locations. According to the investigators of this study, variations in the timing between mammograms and the taking of blood samples and incomplete controlling for confounding variables across the four sub-studies may have affected the results.

An important prospective study undertaken by Verheus et al. (2007)[5] examined the menopausal transitional change in mammographic density with premenopausal IGF-I levels. These investigators followed up 684 premenopausal women from the PEPI-Cancer and Nutrition cohort through to menopause. They found that women with higher premenopausal IGF-I levels had smaller increases in non-dense area and a slightly smaller decrease in dense area during menopause, resulting in higher breast density after menopause. This suggests that postmenopausal mammographic density is dependent on premenopausal levels of IGF-I. This study (not included in **Table A7.1**) did not find an association between premenopausal IGF-I levels and mammographic density.

Appendix 7: Evidence of an association between IGF-I and mammographic density

Overall the evidence for an association between mammographic density and IGF-I is still inconclusive, but based on the larger of the studies summarised in **Table A7.1,** it appears that a positive association is likely, particularly in premenopausal women. These findings on mammographic density and IGF-I, at least for premenopausal women, are consistent with the effect of IGF-I on the proliferative activity of mammary tissue shown in animal [6-9] and primate[10] studies. The findings are also consistent with the positive association between IGF-I and breast cancer risk described earlier in Chapter 3, Section 3.3.4.3. It is possible that levels prior to menopause, when levels are typically higher, particularly adolescence (see **Figure A7.1**), contribute more to mammographic density than postmenopausal levels. A prospective study by Verheus et al.(2007)[5] suggests that postmenopausal mammographic density is dependent on premenopausal levels of IGF-I. Based on the evidence above, it is plausible that treatment with high-dose estrogens for the treatment of tall stature in adolescent girls could reduce mammographic density. Treated girls have been shown to have reduced levels of IGF-I as described in Chapter 2.

Figure A7.1: Range of plasma IGF-I levels (ng/ml) by age (years) for normal males and females. Sourced from Le Roith (1997)[11]

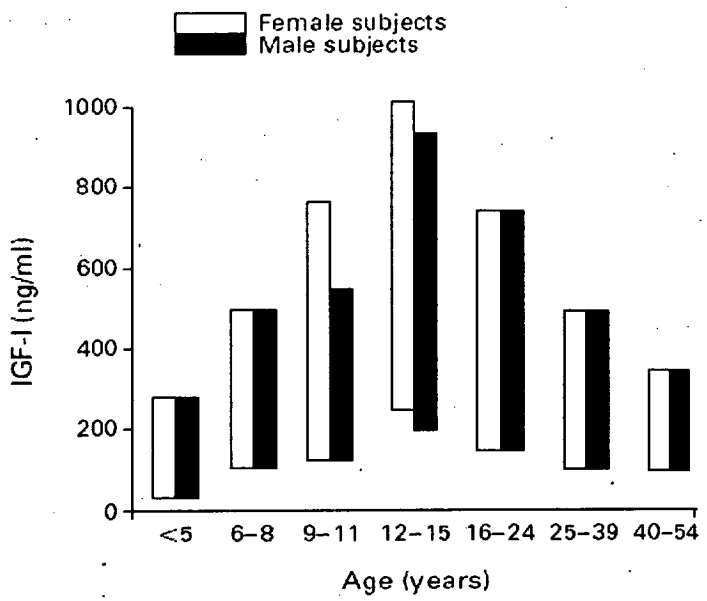

Table A7.1: Cross-sectional studies of the association between plasma IGF-I and mammographic density.

Study	Country	(N) Pre/Post menopause	Density Measure	Hormone	Association	P-value Pre/Postmenopausal	Conditions/Adjustments
Maskarinec et al. 2007[4]	US	525 pre 802 post	% density	IGF-I IGFBP-3 IGF-I/IGFBP-3	0 0 0	0.83/0.57* 0.91/0.67 0.67/0.67	Adjusted: ethnic/location group, age, BMI, digital mammogram, parity, age at menarche, age at first live birth and HRT use, and IGF-I or IGFBP-3 where appropriate.
Aiello et al. 2005[12]	US	88 post	% density	IGF-I IGFBP-3 IGF-I/IGFBP-3	0 0 +	>0.15 >0.29 0.04	Association in former HRT users only. Adjusted: age, body fat, years since menopause, ethnicity.
dos Santos Silva et al. 2006 [13]	UK	215 pre 241 post	% density	IGF-I IGF-II IGFBP-3 IGF-I/IGFBP-3	0 0 0 0	>0.15	Adjusted: age, time since blood collection, age at first birth, BMI, waist circumference, smoking habits, past oral contraceptive use and serum levels of IGFBP-3/IGF-I/IGF-II
			Dense area	IGF-I IGF-II IGFBP-3 IGF-I/IGFBP-3	0 0 0 0		
			Non-dense area	IGF-I IGF-II IGFBP-3 IGF-I/IGFBP-3	0 0 0 0		

Study	Country	(N) Pre/Post menopause	Density Measure	Hormone	Association	P-value Pre/Postmenopausal	Conditions/Adjustments
Boyd et al. 2002[14]	Canada	193 pre 189 post	% density	IGF-I IGFBP-3	+/0 0/0	0.03/0.48* 0.95/0.57	Adjusted: age, waist, and IGF-I or IGFBP-3 whichever applicable.
			Dense area	IGF-I IGFBP-3	+/0 0/0	0.05/0.43 0.95/0.28	
Diorio et al. 2005[2]	Canada	783 pre 792 post	% density	IGF-I IGFBP-3 IGF-I/IGFBP-3	+/0* -/0 0/0	0.02/0.37 0.0005/0.72 0.06/0.41	Adjusted: age, BMI, IGF-I, or IGFBP-3 if applicable
Maskarinec et al. 2003[15]	US	240 pre	% density	IGF-I IGFBP-3 IGF-I/IGFBP-3	+ – +	0.06/0.01 0.02/0.09 0.03/0.007	Adjusted: age, BMI, ethnicity, year of lab analysis, family history of breast cancer, reproductive variables and IGF-I or IGFBP-3 (if applicable
			Dense area	IGF-I IGFBP-3 IGF-I/IGFBP-3	0 0 0	0.09/0.06 0.44/0.73 0.15/0.09	
			Non-dense area	IGF-I IGFBP-3 IGF-I/IGFBP-3	0 +/– –	0.13/0.07 0.004/0.03 0.02/0.02	
Bremnes et al. 2007[1]	Norway	997 post	% density	IGF-I IGFBP-3 IGF-I/IGFBP-3	+ 0 +	0.03/0.03 (P for trend)‡ 0.88/0.55 (P for trend) 0.03/0.06 (P for trend)	Adjusted: age, BMI, number of children, age at menopause, and HT use.
			Dense area	IGF-I IGFBP-3 IGF-I/IGFBP-3	+ 0 +	0.03/0.04 (P for trend) 0.75/0.46 (P for trend) 0.05/0/07 (P for trend)	No association among current HRT users.

Study	Country	(N) Pre/Post menopause	Density Measure	Hormone	Association	P-value Pre/Postmenopausal	Conditions/Adjustments
Johansson et al. 2008[16]	Italy	226 post	% density	IGF-I IGFBP-3 IGF-I/IGFBP-3	0 0 0	0.43 0.10 0.25	Adjusted: age, BMI.
Byrne et al. 2000[17]	US Canada	65 pre 192 post	% density	IGF-I IGFBP-3 IGF-I/IGFBP-3	+/0* –/ 0 +/0	0.007/0.92* 0.07/0.52 0.004/0.83	Adjusted: Age, alcohol intake, batch, BMI, and age at first birth (for postmenopausal) and IGF-I or IGFBP-3 (if applicable)
Lai et al. 2004[18]	Canada	206 pre 206 post	% density	IGF-I IGFBP-3 IGF-I/IGFBP-3	0 0 0	0.9/0.5* 0.4/0.8 0.8/0.5	Multivariable adjusted.

*Premenopausal /postmenopausal results

† non HRT users ‡former HRT users/never HRT users

IGFBP-3 and mammographic density

Many of the studies that examined the association between IGF-I and mammographic density, also examined insulin-like growth factor binding protein-3 (IGFBP-3), and the ratio between the two. IGFBP-3 is the most abundant of the six growth factor binding proteins (See **Table A7.1** above). It is responsible for binding >95% of circulating IGF-I[11]. IGFBP-3 reduces the availability of IGF-I by preventing the factor from interacting with the receptors. It is believed that dissociation allows IGF to leave the circulation and reach target tissues. Because this dissociation is highly regulated, and binding prolongs the half-life of IGF-I its biological response. IGFBP-3 might also have direct actions, independent of IGF-I. For example, IGFBP-3 has been reported to inhibit breast epithelial cell growth[19]. This action has led to the suggestion that IGFBP-3 might act as a tumour suppressor[19].

Maskarinec et al.(2003)[15], Byrne et al. (2000)[17] and Diorio et al. (2005)[2] observed an inverse association between IGFBP-3 and percent mammographic density (see **Table A7.1**). The other four studies in the table that examined the association with IGFBP-3 did not observe an association. The study by Diorio et al., found that women with a combination of high IGF-I and low IGFBP-3 had higher levels of percent breast density. Many of the studies examined the IGF-I/IGFBP-3 ratio. It is suggested that a high ratio, indicating high IGF-I to low IGFBP-3, would also demonstrate a positive association with mammographic density. Among the studies that examined the association between the IGF-I/IGFBP-3 ratio with percent mammographic density, five observed no association while four observed a positive association (one of which was only for premenopausal women only).

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Appendix 8: Current evidence of an association between childhood anthropometric growth variables and mammographic density.

APPENDIX 8

Current evidence of an association between childhood anthropometric growth variables and mammographic density

This appendix reviews the evidence relating to the association of birthweight, birth-length, and other childhood anthropometric parameters on mammographic density.

Birthweight and birth-length

It is possible that girls treated with high-dose estrogens had a different birthweight and/or birth-length than girls who were not treated. Some studies have indicated associations between these anthropometric variables and mammographic density and breast cancer. A review of these studies is presented below.

Birthweight and mammographic density

Six studies have examined the effect of birthweight on adult mammographic density (see **Table A8.1**). Ekbom and colleagues (1995)[1] found no association between Wolfe grade mammographic density and increasing birthweight (P for trend=0.53) in their cross-sectional study of 370 Swedish women. Similarly, McCormack and colleagues (2003)[2] found no meaningful association between birthweight and Wolfe grade, OR 1.03 (95% CI: 0.93 to 1.14), in their large cross-sectional study (n=1298). These findings are supported by Jeffreys et al. (2004)[3] when they examined the association between birthweight and percent mammographic density in 628 Scottish women (≥25% versus <25%) (n=628) (adjusted P trend=0.82).

In contrast to the findings of the three studies above, Cerhan et al. (2005)[4] (**Table A8.1**) reported a positive association between birthweight and percent mammographic density, measured by a computer assisted thresholding technique. However, a strong association was only observed in postmenopausal women (P trend.<0.01) with an adjusted mean percent density of 17.1% for birthweight <2.95 kg and 21.0% for birthweight ≥3.75 kg. A similar association was observed for dense area. This association was not apparent in premenopausal women (P for trend=0.16). Similarly, Tamimi et al. (2009)[5] using the computer assisted thresholding technique and birth records, found a positive association between birthweight and percent density in postmenopausal women. A later study by Jeffreys et al. (2008)[6] using the volumetric method of measurement identified a quadratic relationship.

Table A8.1: Summary of studies that have examined the association between mammographic density and birthweight.

Study	N	Age (years)	Measure	Outcome Scale	Results
Ekbom et al. (1995)[1]	370	40–74	Wolfe grade*	Binary P2 & DY (high risk) vs. N1 & P1 (low risk)	No association P for trend 0.53
McCormack et al. (2003)[2]	1294	53	Wolfe grade	Three binary outcomes: DY vs. P2, P1 N1, DY, P2 vs. P1 N1, DY, P2 P1 vs. N1.	No association Common OR 1.03 (95% CI: 0.93 to 1.14) for each SD increase in birthweight for each binary outcomes
Jeffreys et al. (2004)[3]	628	59	% density	Binary variable: ≥25% vs. <25% dense	No association P for trend 0.82
Cerhan et al. (2005)[4]	940	60.4	% density and dense area	Continuous N.B. Not transformed – assumption that data is normally distributed.	Positive association for postmenopausal only P for trend <0.01
Jeffreys et al. (2008)	490	54.1	Volum-etric % density.	Categorical.	Quadratic pattern: lowest risks in women born under 2.5 and over 4 kg.
Tamimi et al. (2009)[5]	893	61.2	% density	Continuous Binary variable: (50% cutoff)	Linear trend p=0.02 Binary: birthweights 3001–3500g had higher odds of having high mammographic density OR: 2.9 (95% CI: 1.1 to 7.9) compared with birthweights >4000g.

* Wolfe Grade: N = fatty radiolucent breast, P1 and P2 greater levels of prominence of fibroglandular tissue (hence density), DY = dense sheets of fibroglandular tissue.

Birthweight and breast cancer

A recent review and meta-analysis (Xue and Michels,2007)[7] updated from a previous analysis[8] including 11 cohort and 17 case-control studies observed an overall increased risk of breast cancer in women who had a higher birthweight regardless of menopausal status. They calculated a summary estimate of RR 1.15 (95% CI:1.09 to 1.21). These findings are supported by two earlier reviews (Okasha et al., 2003[9] and Forman et al., 2005[10]). The first of these did not differentiate between menopausal status while the latter observed an increased risk with increasing birthweight only in premenopausal women.

Birth-length and mammographic density

Fewer studies have examined the association between birth-length and mammographic density or breast cancer. As well as birthweight as reported above, Ekbom et al. (1995)[1], found no association between birth-length and Wolfe grade mammographic patterns (P for trend 0.52). Nor did Tamimi et al. (2009)[5] with the continuous measure of percent density measured using a computer thresholding technique. No other reported study has examined the association between birth-length and mammographic density. Since many of the risk factors for breast cancer are shared with mammographic density, reports of associations between birth-length and breast cancer are of relevance.

Birth-length and breast cancer

Two reviews of birthweight and breast cancer mentioned earlier also examined the association between birth-length and breast cancer risk. The meta-analysis reported by Xue and Michels (2007)[7], involved four case-control and four cohort studies and found an increased risk of breast cancer with increasing birth-length. They calculated a summary estimate of RR 1.28 (95% CI: 1.11 to 1.48).

Childhood height

It is likely that girls treated with high-dose estrogens were taller at different stages of childhood than the girls who were not treated. Some studies have indicated associations between height at different ages in childhood and mammographic density or breast cancer. A review of these studies is presented below.

Childhood height and mammographic density

Two studies examined the association between childhood height and mammographic density with both finding positive associations between the two variables. McCormack et al. (2003)[2] found height at ages two and 11 had significant but opposing associations with higher Wolfe grade density; adjusted OR 1.13 (95% CI: 1.01 to 1.26) (p=0.03) (n=1033), and OR 0.89 (95% CI: 0.80 to 1.00) (p=0.04) (n=1090), respectively, however, no significant association was observed for ages 4, 7, or 15 years. In contrast, Sellers et al. (2007)[11] observed a positive association between height at ages seven (p<0.001), 12 (p<0.001), and 18 years (p<0.001) with percent density (n=1893). The minimum adjusted mean difference in percent density between the tallest and shortest girls was 3%, while the maximum difference was 7%.

Childhood height and breast cancer

A number of studies have examined the association between childhood height and breast cancer risk and report conflicting results (**Table A8.2**).

Le Marchand et al. (1988)[12] undertook an age matched nested case-control study of 38,084 women born between 1918 and 1943 (607 cases of breast cancer), on whom information about weight and height had been recorded in Hawaii in 1942–1943. They found no association between height at age 10–14 years and risk of premenopausal or postmenopausal breast cancer.

Herrinton and Husson (2001)[13] undertook a case-control study of 214 breast cancer cases (predominantly premenopausal) and 214 matched controls and observed a positive association between height at 15–18 years (tall for age vs. short for age) and breast cancer risk, OR 2.2 (95%

CI: 1.1 to 4.3) but did not find an association with height at earlier years (9–11 years), OR 1.0 (95% CI: 0.5 to 1.8), which is consistent with the larger case-control study by Le Marchand and colleagues described above.

Whitely et al. (2009)[14] undertook a study of a sub-population of the Boyd-Orr cohort (2009)[14] that involved 2960 women followed-up for 59 years (69 cases of breast cancer). They similarly found no increased risk of breast cancer with increasing childhood height (1 sd increase) OR 1.07 (95% CI: 0.81 to 1.42) adjusted for age at measurement.

In contrast to the studies above, one case-control and two cohort studies support a positive association between childhood height and breast cancer risk. Hilakivi-Clarke et al. (2001)[15] studied the childhood height records of 3447 women born in Finland during 1924–1933 and found that at each age between 7–15 years, the girls who later developed breast cancer as an adult were on average taller. Unadjusted hazard ratios rose across the range of height at age seven years (p=0.01).

Swerdlow et al. (2002)[16] undertook a case-control study of twins pooled from four studies across Europe. This study involved 400 cases and 400 co-twins who had not yet developed breast cancer. They found that risk of premenopausal breast cancer was increased for the co-twin who was taller at age 10 years; OR 1.27 (95% CI: 0.95 to 1.70). They did not examine the risk of postmenopausal cancer in this cohort of women.

Ahlgren et al. (2006)[17] undertook a retrospective cohort study of 117,415 Danish women (3,333,359 person-years of follow-up, 3,340 cases of breast cancer) and found height at eight years to be positively associated with breast cancer risk: adjusted RR 1.11 (95% CI: 1.07 to 1.15). This relative risk is not unlike that observed by Whitley et al. (2009)[14] in the Boyd-Orr study reported above, which had too few breast cancer cases.

Table A8.2: Height at certain childhood ages and breast cancer risk.

Study	Country	Design	N	Age (year)	Results	Adjustments
Le Merchand et al.(1988)[12]	US	Case-control	38,084	10–14	p>0.01	Age matched
Herrinton & Husson (2001)[13]	US	Case-control	428	3–5	OR 0.8 (95% CI: 0.3 to 1.9)*	Age matched
				6–8	OR 0.9 (95% CI: 0.5 to 1.8)*	
				9–11	OR 1.0 (95% CI: 0.5 to 1.8)*	
				12–14	OR 1.7 (95% CI: 1.1 to 2.8)*	
				15–18	OR 2.2 (95% CI: 1.1 to 4.3)*	
Hilakivi-Clarke et al. (2001)[15]	Finland	Cohort	3,447	7	HR 1.9 (95% CI: 1.1 to 3.1)†	
				15	HR 1.9 (95% CI: 1.2 to 3.2)§	
Swerdlow et al. (2002)[16]	Europe	Aggregate of	800	7	OR 1.21 (95% CI: 0.91 to 1.61)¶	Twin matched
		4 case-		10	OR 1.27 (95% CI: 0.95 to 1.70)¶	Parity
		controls		20	OR 1.22 (95% CI: 0.94 to 1.58)¶	Weight
De Stavola et al. (2004)[18]	UK	Cohort	1,782	2	OR 1.16 (95% CI: 0.88 to 1.54)**	
			1,944	4	OR 1.12 (95% CI: 0.86 to 1.46)	
			1,925	7	OR 1.30 (95% CI: 0.99 to 1.71)	
			1,862	11	OR 1.16 (95% CI: 0.88 to 1.53)	
			1,689	15	OR 1.33 (95% CI: 0.97 to 1.80)	

Study	Country	Design	N	Age (year)	Results	Adjustments
Ahlgren et al. (2006)[17]	Denmark	Cohort	117,415	8	RR 1.11 (95% CI: 1.07 to 1.15)	Birthweight Age at menarche
Whitley et al. (2009)[14]	UK	Cohort (59 year follow-up)	2,960	2–14	OR 1.07 (95% CI: 0.81 to 1.42)	Age at height measurement

* Tall for age vs. small for age

† ≥123 cm vs. <114.5 cm

§ ≥163 cm vs. <153 cm

¶ Premenopausal women. Postmenopausal women not examined.

** Estimated univariable odds ratios for breast cancer according to a one-standard-deviation increase in childhood height at different ages.

Childhood BMI

It is possible that untreated girls had a higher BMI during adolescence compared with treated girls. BMI in childhood is highly correlated with BMI in adulthood[19-21] and untreated women had a higher BMI, on average, than treated women. If childhood BMI and mammographic density are associated, this might explain or influence the difference in mammographic density observed between treated and untreated women. The following section reviews the studies that have reported on the association between childhood BMI and mammographic density and breast cancer.

Childhood BMI and mammographic density

McCormack et al. (2003)[2] and Sellers et al. (2007)[11] in their studies reported above, also examined the association between childhood BMI or weight and mammographic density. McCormack and colleagues[2] found a lower odds ratio for a standard deviation increase in BMI at any age during childhood (or adult life) after controlling for breast size and BMI at mammography (See **Table A8.3**).

Table A8.3: Odds ratios for standard deviation increase in BMI by age controlled for breast size and BMI at mammography.

Age	N	Odds Ratio (95%CI)	P-value
2	994	0.88 (0.79 to 0.98)	0.02
4	1093	0.89 (0.80 to 0.99)	0.03
7	1074	0.72 (0.64 to 0.80)	<0.001
11	1079	0.56 (0.49 to 0.64)	<0.001
15	989	0.56 (0.49 to 0.64)	<0.001
26	1129	0.62 (0.53 to 0.75)	<0.001
43	1209	0.54 (0.43 to 0.68)	<0.001

Sellers et al.[11] likewise found weight (p=0.005) and adiposity (p=0.005) at age 12 years to be inversely associated with adult percent density. This effect remained following adjustment for current BMI. And similarly, Samini et al. (2008)[22], in their cross sectional analysis of 1398 women

in the Nurses Health Study, observed childhood body fatness to be inversely associated with percent mammographic density (p=0.0004) (adjusted r= – 0.19 for premenopausal and –0.15 postmenopausal). Confidence intervals were not reported.

Childhood BMI and breast cancer

A number of studies have examined the association between childhood weight/BMI with breast cancer risk later in life.

Okasha et al. (2003)[9] undertook a review of 35 case-control and seven cohort studies on the association between childhood weight and breast cancer risk. There was no consistent pattern of association between weight in childhood or adolescence and risk of breast cancer. Case-control studies suggested a reduced risk of breast cancer among women who were overweight in early life, while cohort studies were inconsistent with early life BMI appearing to have a protective significant effect (n=3 studies), or no effect (n=4). Two of the latter studies had too few breast cancer cases ranging from eight to 69.

Three studies that have been published since the review by Okasha et al.(2003)[9] all support an inverse association between childhood BMI or weight and later breast cancer risk. In the multi-centred pooled case-control study of twins by Swerdlow et al. (2002)[16] reported above and summarised in **Table A8.2**, risk of premenopausal breast cancer was increased for the co-twin who was less obese at age 10 years; OR 1.44 (95% CI: 1.08 to 1.91). This association was not statistically significant for weight at seven years OR 1.29 (95% CI: 0.97 to 1.69).

Cerhan et al. (2004)[23] showed family history of breast cancer to modify the effect of obesity in early adolescence (e.g. 12 years) on breast cancer risk later in life. Of those who had a family history of the disease, the risk of breast cancer was increased in those with below average weight at age 12 (RR 1.55, 95% CI: 0.67 to 3.64) and strongly increased in those with above average weight (RR 4.25; 95% CI: 1.71 to 10.5) compared to those with average weight. In contrast, among those without a family history of breast cancer there was only a weak positive association for those with below average weight (RR=0.75, 95% CI: 0.26 to 2.16), compared to those with average weight. This study, similar to that by Swerdlow et al. (2002)[16] above, relied on self-reported recall of weights at childhood.

A large cohort study that used direct measures of childhood weight was undertaken by Ahlgren et al. (2004)[24] (separately reported in 2006[17]). Their cohort study of 117,415 Danish women (3340 breast cancer cases) found BMI measured at age 8, 10, 12 and 14 years of age to be inversely associated with the risk of breast cancer, however, only the association with BMI at 14 years remained after adjustment for age at menarche. The adjusted attributable risk of BMI at 14 years of age was 15% and the relative risk of breast cancer for each unit increase in BMI = 0.97 (95% CI: 0.96 to 0.98). Age at menarche is inversely associated with BMI at childhood[25]. Adjusting for age at menarche might have removed any 'true' association between BMI at ages 8, 10 and 12 and mammographic density if age at menarche and BMI were collinear.

Childhood weight/BMI velocity

Girls treated with high-dose estrogens commonly experienced rapid increases in weight gain following treatment[26]. Evidence suggests that change in weight gain is associated with mammographic density as described below.

Childhood weight/BMI velocity and mammographic density

McCormack et al. (2003)[2] examined the effect of BMI velocity at different age intervals with Wolfe grade breast parenchymal patterns. They found that an increase in BMI during any period up to age 43 years was associated with reduced odds of a greater Wolfe grade with larger inverse associations in the preadolescent years (7–11 years) (See **Table A8.4**).

Table A8.4: Odds ratios for standard deviation increase in BMI by age controlled for breast size and BMI at mammography.

Age	N	Odds Ratio (95%CI)	P-value
2–4	911	0.56 (95% CI: 0.49 to 0.64)	<0.001
4–7	950	0.62 (95% CI: 0.54 to 0.71)	<0.001
7–11	975	0.50 (95% CI: 0.43 to 0.59)	<0.001
11–15	929	0.72 (95% CI: 0.63 to 0.83)	<0.001
15–26	904	0.72 (95% CI: 0.63 to 0.83)	<0.001
26–36	1054	0.86 (95% CI: 0.75 to 0.99)	0.03
36–43	119	0.85 (95% CI: 0.75 to 0.98)	0.02
43–53	1188	0.94 (95% CI: 0.82 to 1.07)	0.33

Childhood BMI/weight velocity and breast cancer

The findings reported above by McCormack and colleagues on weight velocity/gain and mammographic density are consistent with the association observed between BMI velocity at ages 2–4 years and breast cancer risk in the retrospective cohort study by De Stavola et al. (2004)[18]. They observed an odds ratio of 0.68 (95% CI: 0.48 to 0.83) for BMI velocity (kg/height $(m)^2$ /year) measured at 2–4 years. However no association was observed in the 4–7 or 11–15 age categories.

Childhood height velocity

Linear growth velocity is another parameter of interest to this study. Treated and untreated women might have had different rates of pre-treatment or post-treatment linear growth velocity.

Differences in pre-treatment rates of growth velocity would be expected if treated and untreated girls differed in height at particular ages. Untreated girls might have reached maturity earlier and therefore reached their peak velocity earlier. Peak height velocity typically occurs one year prior to menarche. Less growth potential was one of the main reasons untreated girls were not

treated. It is possible, therefore, that they have reached mean peak height velocity earlier than treated women. On the other hand, treated women may have been taller at particular childhood ages than untreated women. If so, it is likely that height velocity was greater in treated girls compared with untreated girls.

Post-treatment differences in height velocity are also likely. It has been reported that treatment with high-dose estrogens for the curtailment of growth in adolescent girls reduces height velocity[27].

If any childhood height velocity or the age at which it reaches its peak is associated with mammographic density, then differences in these variables between treated and untreated women might explain the association observed between treatment and mammographic density reported in Chapter 5. Studies that have examined the association between childhood height velocity and mammographic density and breast cancer risk are summarized below.

Childhood height velocity and mammographic density

A positive association between height velocity (cm/yr) from 15 years to adulthood, and Wolfe grade mammographic patterns was found by McCormack et al. (2003)[2]. The odds of having had a higher grade of mammographic pattern was increased with every standard deviation increase in height velocity OR 1.16 (95% CI: 1.03 to 1.29) (p=0.01). Increased odds were also observed for other ages, but these were not statistically significant [11–15 years, OR 1.11 (95% CI: 0.99 to 1.24) (p=0.08) (n=950) and 7–11 years OR 1.00 (95% CI: 0.86 to 1.15) (p=0.96) (n=1,030)]. In contrast, a reduced odds of higher Wolfe grade was observed for a standard deviation increase in height velocity between ages 2–5 years (OR=0.85, 95% CI: 0.76 to 0.96).

Childhood height velocity and breast cancer

While only one study has examined the association between height velocity and mammographic density, a number of studies have examined the association with breast cancer risk. Of these was by Berkey et al. (1999)[28]. They modelled peak growth velocity from the three variables: age at menarche, body fatness at age 10 years and final height. Women who participated in the Nurses Health Study (n= 65,140) were followed up for 16 years. Of these, 806 developed premenopausal breast cancer and 1485 were diagnosed with postmenopausal breast cancer. They found that the women who were in the two highest quintiles of linear peak height velocity in adolescence had a 30% (adjusted RR 1.31; p=0.001) and 40% (adjusted RR 1.40, p=0.001) higher risk of pre and postmenopausal breast cancer, respectively, compared to those in the lowest quintile. A limitation of this study is that peak height velocity was modeled using age at menarche, adult height and body fatness at age 10, that latter was self-reported as an adult using diagrams of body shapes.

De Stavola et al. (2004)[18] analysed prospective growth data from a British cohort of 2,547 girls (59 breast cancer cases) and found that height velocity at ages 4–7 years was associated with an increased risk of breast cancer (for a one-standard-deviation increase, OR 1.54, 95% CI: 1.13 to 2.09). An increased risk was also observed for increasing height velocity between the ages of 11–15 years but this was not statistically significant (OR 1.29, 95% CI: 0.97 to 1.71).

Ahlgren et al. (2006)[17] in their study of 117,415 Danish women observed an increased breast cancer risk with large increases in height during puberty (8–14 years of age). The adjusted relative risk for breast cancer was 1.17 (95% CI: 1.09 to 1.25) for each 5cm increase in height during this age period. These investigators also examined the age at peak height velocity in relation to breast cancer risk. They calculated an adjusted relative risk of 0.94 (95% CI: 0.91 to 0.97) per one-year increase in age at peak growth and an adjusted attributable risk of 9% for earlier age at peak growth.

Age at maximum height

A few studies have examined the association between the age at which maximum height was achieved and mammographic density or breast cancer risk. The age at which maximum height is achieved is not a pre-treatment parameter since treatment might have influenced this, however, if

this variable influences the association observed between treatment and mammographic dense area, it might tell us something about the mechanism of action of treatment on dense area.

Age at maximum height and mammographic density

Sellers et al. (2007)[11] examined the association between age at which participants (n=1298) stopped getting taller and mammographic density measured by a computer assisted thresholding technique. They found no association (P for trend 0.10) between the two variables.

Age at maximum height and breast cancer

Li et al. (1997)[29], in a study of 747 women from the United States, diagnosed with invasive breast cancer before 48 years and 961 controls, observed a trend of decreasing risk of breast cancer with increasing age of maximum height attainment. A 30% reduction in the risk of breast cancer was calculated for women who reached their maximum height ≥18 years of age compared with women who reached their maximum height ≤13 years of age (OR 0.7, 95% CI: 0.5 to 1.0) (P for trend=0.02).

Baer et al. (2006)[30] examined the association between age at maximum attained height with premenopausal breast cancer in 37,572 premenopausal women participating in the Nurses Health Study II. They found no overall increased risk of breast cancer in women who attained their maximum height ≥18 years of age compared to those whose age at maximum height was attained before 14 years RR 0.96 (95% CI: 0.66 to 1.30) (P for trend = 0.65). A limitation of both this and the study above by Li and colleagues is that age at which maximum height was attained was self-reported many years after the event.

Li et al. (2007)[31], again assessed the relationships between age at which maximum height was attained and risk of different types of breast cancer in a prospective cohort of 27,536 women who had previously participated in the VITamins and Lifestyle study. Women who reached their maximum height ≤12 years of age had a 1.4 fold (95% CI: 1.0 to 1.8) increased risk of breast cancer compared to those who reached it ≥17 years of age (P for trend 0.04). This association, however, was limited to estrogen receptor-negative tumours HR 1.9 (95% CI: 1.0 to 3.9). Age at maximum height was again self-reported.

Bone age

Age at menarche is positively associated with breast density[32] suggesting that treated women who matured later are likely to have higher density than untreated women. Bone age is a predictor of age at menarche[33,34] and was measured in both treated and untreated girls at first assessment. Girls with a bone age greater than their chronological age have a greater level of skeletal maturity at age at measurement and less growth potential compared to girls with a bone age lower than their chronological age. It is known that a large number of girls were not treated because they had little growth potential at time of height assessment, indicating that they had greater bone maturity or bone age for a given chronological age[35]. It is possible then that this difference in bone age at time of measurement may explain differences in dense area between treated and untreated women, independent of treatment. A review of the literature failed to find any studies reporting the association between bone age and mammographic density or breast cancer risk.

Overview of research literature and relevance to the study

The review above suggests an association between a number of childhood growth parameters and mammographic density and/or breast cancer risk. Only one study[1] had examined the association between birth-length and mammographic density. This study found no association between the two variables, however breast cancer risk is different. A meta-analysis[7] of studies demonstrated greater consistency in the findings across studies and calculated a significant positive association between both birthweight and birth-length with breast cancer risk. Childhood height and growth velocity has been shown to be associated with both mammographic density[2,11] and breast cancer risk[15-17], though the ages at which these associations exist differed across the studies. Some studies, in contrast, found no association with breast cancer risk[12-14].

Evidence is more consistent for an association between childhood BMI and mammographic density and breast cancer risk, particularly in later studies. Studies suggest that earlier age at peak height velocity[17] and age at which maximum height is attained[29,31] (but not that by Baer et al. (2006)[30] is associated with breast cancer risk, but the studies that examined the latter association relied on self-reported measures of age at which maximum height was attained. No association was observed in the one study that examined age at maximum height and mammographic density[11].

Box A8.1: Key Points from the literature: Appendix 8

KEY POINTS FROM THE LITERATURE: APPENDIX 7

- Evidence suggests an association between a number of childhood growth parameters and mammographic density and or breast cancer risk.
- The only study that examined the association between percent density and birthweight and had used a continuous measure of percent density found a positive association in postmenopausal women only.
- The one reported study that has examined the association between birth-length and mammographic density found no association.
- Evidence suggests a positive association between both birthweight and birth-length with breast cancer risk.
- Childhood height and growth velocity has been shown to be associated with mammographic density. Studies that examined the association between growth velocity and breast cancer risk are less consistent in their findings.
- Earlier age at peak height velocity and age at which maximum height is attained have cautiously been associated with breast cancer risk.
- Childhood BMI has been consistently shown to be inversely associated with mammographic density and breast cancer risk.
- Only one study examined the association between age at maximum height and mammographic density. It relied on self reported age at maximum height as an adult.
- Pre-exposure and variables directly associated with both the exposure and outcome variables could be potential confounders for the association between treatment with high-dose estrogens in adolescence and mammographic density.
- Treatment induced changes (e.g. BMI) that are directly associated with mammographic density might mediate any association between treatment with high-dose estrogens in adolescence and mammographic density.

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APPENDIX 9

Follow-up 2 consent form, study invitation letter, information brochure and mammogram release form

Appendix 9: Follow-up 2 consent form, study invitation letter, information brochure and mammogram release form.

Menzies Research Institute

TALL GIRLS BREAST DENSITY STUDY

CONSENT FORM

1. I have read and understood the 'Information Sheet' for this study.

2. The nature and possible effects of the study have been explained to me.

3. I understand that the study involves the following procedures:

   - A telephone interview about myself, my health and medical history;
   - Providing permission to access the x-ray film of a mammogram if I have had one in the past two years,
   - If I have not had a mammogram in the past two years, to have one at a convenient BreastScreen centre as part of a breast cancer screening check and give the investigators permission to access the film.
   - Allowing BreastScreen to provide the study investigators with information about my BreastScreen attendances including HRT use at the time of my mammogram.

4. I understand that the following risks are involved:

   - Screening mammography for breast cancer is not 100% accurate. This means that sometimes women may be brought back for further tests, which would otherwise not have been necessary. It also means that for a very small number of women the screening mammogram may not find all cancers.
   - Screening mammograms involve exposing women to a small amount of radiation. The level of radiation women receive is low, similar to that from other x-rays people commonly have.
   - Compressing the breast during the mammogram may cause discomfort.
   - There is a small possibility that the original mammogram may be lost in transit for analysis.
   - Some BreastScreen services or private radiology clinics at their discretion, choose to copy the original film prior to sending it to us, however the copy may not be as good a quality as the original.

5. I understand that all research data will be securely stored on the University of Tasmania premises until no longer required, at which time it will be destroyed).

6. Any questions that I have asked have been answered to my satisfaction.

7. I agree that research data gathered for the study may be published (provided that I cannot be identified as a participant).

8. I understand that my identity will be kept confidential and that any information I supply to the researcher(s) will be used only for the purposes of the research.

9. I agree to participate in this investigation and understand that I may withdraw at any time without any effect, and if I so wish, may request that any personal data gathered be withdrawn from the research.

Name of participant:
Signature of participant: _____ Date:

_____

22 September 2010

«ID_number»

«First_name» «Surname»
«MailAddress1»
«MailAddress2»
«MAILSUBURB» «MAILSTATE» «MailPostcode»

Dear «First_name»

You may remember that in 2002 you participated in the Tall Girls Study by completing a postal questionnaire and telephone interview. A total of 836 women contributed to this research which resulted in some important findings on the long-term effects of treatment for tall stature. We remain very grateful for your contribution to this research. A summary of the findings can be found at **http://www.latrobe.edu.au/mchr/tallgirls.html**.

When you completed the telephone interview, you indicated that you would be willing to be contacted again for further research. We are now inviting women who were **treated or assessed and not treated** for tall stature during adolescence, and who are aged 40 years and over, to participate in a new study - the *Tall Girls Breast Density Study*. It is important that untreated as well as treated women participate in this study.

This study is being conducted by Associate Professor Alison Venn (now of the Menzies Research Institute, University of Tasmania) and PhD student Helen Jordan, in collaboration with Associate Professor Anne Kavanagh and Associate Professor Dorota Gertig (University of Melbourne).

The Tall Girls Breast Density Study aims to find out whether oestrogen treatment to reduce the adult height of tall girls has had any long-term effects on breast tissue. One of the features of breast tissue is the proportion of dense tissue that appears on a breast x-ray (mammogram). This feature, referred to as mammographic density, has become recognised as a risk factor for breast cancer. Mammographic density is affected by hormones such as oestrogen, however, it is not known whether hormone levels in adolescence have any long-term effects on the breast.

If you agree to participate in this study, we will need to have access to your mammogram. If you have had a mammogram in the last two years, we would like your permission to borrow your mammogram from the service where you had the procedure or where it is currently being held.

If you have <u>not</u> had a mammogram in the past two years, we would like you to make an appointment to have a mammogram at a convenient BreastScreen centre. BreastScreen
will send us the mammogram after they have performed their usual breast cancer screening tests and have informed you of the result. The attached brochure explains the procedure involved in having a breast cancer screening mammogram.

We will arrange to get your mammogram from the BreastScreen centre or medical practice holding it: you will not need to organise this. We will need to retain the mammogram for approximately one month while we take measurements of breast density.

To help us retrieve your mammogram, we would also like your permission for BreastScreen to provide us with information about your BreastScreen attendances including HRT use at the time of mammogram. This will assist us to retrieve the most relevant mammogram.
We would also like to interview you by telephone, at a time convenient to you to ask questions about a range of factors that may be associated with your breast density. This interview will take approximately 20 minutes.

You are under no obligation to be part of the study and should you decide to participate, you are free to withdraw from the study at any time. Your confidentiality will be maintained and personal information will not be released without your consent.

Enclosed is an information sheet describing the study, as well as consent and mammogram release forms. We ask that you read the information sheet carefully and ask us any questions you may have about the study prior to making a decision about participating.

If you are willing to participate in this study, please fill in the yellow consent and mammogram release forms and mail them back in the enclosed reply-paid envelope as soon as possible. The white consent form is for your records. If we do not hear from you, you may receive a call from us to check that you have received this letter. Please note that you do not need to have had a mammogram, or to have made an appointment for a mammogram, before returning the consent form

If you are willing to participate, one of our research staff will telephone you to answer any questions you have about the study and to arrange a convenient time for you to be interviewed.

If you have any questions, please contact Emma Stubbs on (03) 6226 4709.

Yours sincerely

Associate Professor Alison Venn
CHIEF INVESTIGATOR

ENCL.

What about my privacy?

The information you provide for the study will be kept confidential and held at the Menzies Research Institute at the University of Tasmania. The data collected will be used only for the purposes of this study. Data will be coded and entered onto a computer without your name. The results of the research will be published in a form that will not allow individuals to be identified.

If you attend BreastScreen for a mammogram, you will be treated in the same way as all other women in the community attending the service and will be required to provide them with information about yourself. The BreastScreen service will allow us to access your mammogram but the staff at each service might not be aware of this study and they will not know whether or not you were treated as a tall girl.

Who is doing the research?

The study is being conducted by a group of researchers led by Associate Professor Alison Venn (Menzies Research Institute, University of Tasmania), Ms Helen Jordan (PhD candidate at the Menzies Research Institute), Associate Professor Anne Kavanagh and Associate Professor Dorota Gertig (University of Melbourne).

Can I be told about the results?

The overall study results on the effects of hormone treatment for tall stature will be provided to you if you would like this information. We expect to have results of the study in about two years. If you would like to hear about the results, please indicate this when asked in the telephone interview. They will also be made available on our website: www.menzies.utas.edu.au.

The BreastScreen service you attend will provide you with the results of your screening test for breast cancer. Your individual breast density result will not be available to you. While breast density measurement has proven research value, it has not been shown to have clinical benefit for individuals.

What do I do now if I agree to participate?

Complete the two yellow consent forms and mail them back in the enclosed reply-paid envelope. The white consent form is for your records.

We will contact you in the near future to answer any questions you may have about the study and arrange an appropriate interview time with you.

Who do I contact if I have questions or concerns?

If you have any concerns or questions or would like more information, contact Emma Stubbs on (03) 6226 4709 or freecall 1800 638 124.

If at any time you have any concerns about your involvement in the study that the researcher has not been able to answer to your satisfaction, you may contact the Executive Officer of the Human Research Ethics Committee (Tasmania) Network on (03) 6226 2763.

Menzies Research Institute

University of Tasmania
Private Bag 23
HOBART TAS 7001

Phone: (03) 6226 4709
Fax: (03) 6226 7704

Menzies Research Institute

THE TALL GIRLS BREAST DENSITY STUDY

STUDY INFORMATION

What is the study about?

The Tall Girls Breast Density Study aims to find out whether oestrogen treatment to reduce the adult height of tall girls has had any long-term effects on breast tissue. One of the features of breast tissue is the proportion of dense tissue that appears on a breast x-ray (mammogram). This feature, referred to as "mammographic density", has become recognised as a risk factor for breast cancer. Fatty tissue appears dark on a mammogram, while denser tissue appears as light areas. Mammographic density is known to be affected by hormones such as oestrogen. However, it is not known whether hormone levels in adolescence have any long-term effects on the breast.

Some women who were treated with oestrogens to reduce their adult height have been concerned about the possibility of an increased risk of breast cancer. The number of tall girls who have been treated in Australia is too small for us to be confident about detecting any increase in breast cancer risk. However, by looking at mammographic density, we will be able to see if treatment for tall stature is associated with any long-term changes to breast tissue and breast cancer risk.

Currently little is known about the effect of hormones in adolescence on breast cancer risk. This research will tell us whether hormonal exposures during adolescence have any long-term effects on mammographic density and hence breast cancer risk.

Who is eligible to participate?

You are eligible to participate in the study if you are aged 40 years or over, and you participated in the previous Tall Girls Study. It is very important that our study includes tall girls who were assessed but not treated, as well as those who were treated.

How did you get my name?

Your name was obtained from the names of eligible women who consented to participate in the previous Tall Girls Study, and who expressed an interest in being involved in further research.

What exactly do you want me to do?

If you agree to participate in this study, we will ask you to:

- Complete a telephone interview about yourself, your health and medical history.
- If you have had a mammogram in the past two years, we would like you to give us permission to access the x-ray film.
- If you have not had a mammogram in the past two years, we would like you to have one at a convenient BreastScreen centre and give us permission to access the mammogram after all follow-up at BreastScreen is complete. The attached brochure explains the BreastScreen mammogram and follow-up procedure. It is free of charge. The results of the screening mammogram and any appropriate follow up will be made available to you by the BreastScreen service, completely independently of our study. Once you have had your mammogram we would like you to contact us on (03) 6226 4709 or freecall 1800 638 124 to tell us when and where you had your mammogram performed. (Please note that if you have had implants in both breasts we will not ask you to have a mammogram).
- To give BreastScreen permission to provide us with information about your BreastScreen attendances including HRT use at the time of your mammogram. This will assist us to retrieve the most relevant mammogram.

Once we have retrieved and scanned your mammograms, we will send them back, usually within 1 month. Mammograms borrowed from BreastScreen, your doctor or a clinic will be returned directly to the service. Mammograms borrowed directly from you will be returned to you by registered mail. If you need your mammograms at short notice after we have received them, please call us so that we can return them to you quickly.

Do I have to participate?

Participation in the study is entirely voluntary. You are free to withdraw from the study at any time. You are also free to not answer any particular question during the interview. If you do not wish to participate in the study, please let us know by filling out the appropriate box on the consent form (yellow form) and sending it back to us in the enclosed self- addressed envelope.

What's in it for me?

As well as improving understanding of the long-term effects of oestrogen treatment for tall stature, this study will help to increase scientific understanding of how hormones in adolescence influence breast development and longer-term breast cancer risk.

If you have not had a mammogram in the past 2 years, and you agree to participate in this study, you can also expect to receive the benefits of mammographic breast cancer screening.

What are the benefits of mammographic screening?

Having regular screening mammograms is the best way to find breast cancer at an early stage, before it can be felt or noticed. Finding breast cancer early often means that the breast cancer is small, has less likely spread to other parts of the body and is more effectively treated.

Whilst all women are at risk of developing breast cancer, it is far more common as we grow older. It is clear that mammography screening of women over 50 reduces the number of deaths from breast cancer. However, screening has been shown to be less effective for women aged between 40 and 49 years, as breast tissue in younger women tends to be denser. This makes it more difficult to detect small changes in the breast. While the national breast screening program is active in recruiting women in the 50-69 year age group, women aged 40 years and over are eligible to be screened.

What are the risks of participating in this study?

If you have not already had a breast screening mammogram, and you agree to have one as part of this study, you will need to consider the risks associated with screening mammography. While screening mammography can find most breast cancers present at the time of screening, like many other medical tests, it is not 100% accurate. This means that sometimes women are brought back for further tests, which would otherwise not have been necessary. It also means that for a very small number of women the screening mammogram may not find all cancers.

Screening mammograms involve exposing women to a small amount of radiation. The level of radiation received is low, similar to other common x-rays (e.g. chest).

Compressing the breast during the mammogram may cause discomfort. Some women may experience more discomfort than others.

In the course of this study there is a very small risk that an original mammogram could be mislaid or damaged in transit. We can reassure you that all effort will be made to avoid this. The process we are using has been used for another Australian study and to date this study has borrowed and scanned more than 2000 mammograms without incident. As an additional safe-guard, some BreastScreen Services or other radiological services holding your mammogram may, at their discretion, choose to make a copy of the mammogram prior to it being released. The quality of a copy may not be as good as the original and cannot be guaranteed.

Please refer to the information provided by BreastScreen for further details about what you can expect with breast cancer screening.

Appendix 9: Follow-up 2 consent form, study invitation letter, information brochure and mammogram release form

TALL GIRLS BREAST DENSITY STUDY

AUTHORISATION TO RELEASE MAMMOGRAM AND HISTORY OF MAMMOGRAM SCREENING

I _____ _____ _____
Firstname Middlename Surname

Date of Birth ___ / ___ / 19___, hereby authorise:

BreastScreen

or

_____
(name of your doctor, or the clinic at which you mammogram is held

_____
(address of doctor, or the clinic at which your mammogram is held

- to release my **ORIGINAL** mammogram on a temporary basis to Associate Professor Alison Venn, Menzies Research Institute, University of Tasmania, to be scanned for use in the Tall Girls Breast Density Study; and
- for BreastScreen to provide Associate Professor Alison Venn, Menzies Research Institute, University of Tasmania, with a history of my BreastScreen attendances and information about my HRT use at time of each mammogram.

I understand and agree that:

- The mammogram will be used in a research study of Mammographic Density
- My left and/or right cranio-caudal mammography X-Ray will be released to the researchers, for the purpose of this study, but only after all follow-up at BreastScreen is complete, and at no cost to me
- The mammogram will be returned to BreastScreen, the Doctor/Clinic or me as soon as it has been scanned, after about one month
- While all efforts will be made to avoid the loss of the mammogram during handling, there is a very small risk that the mammograms could be damaged or lost in transit
- At their discretion, the BreastScreen service or private service holding the mammogram may choose to make a copy of the mammogram, at no cost to me, before releasing the original to the researchers
- The BreastScreen registry can inform the study co-ordinator that I have attended their service and had a mammogram.

Signature of participant: _____ Date: _____

Telephone Contact:

Please provide your phone number(s) so we can contact you to arrange a suitable interview time:

Phone: _____ (h)_____(w) _____(m)

TALL GIRLS

BREAST DENSITY STUDY

MAMMOGRAM DETAILS

Please fill out if you have had a mammogram in the past two years.

If you are to have a mammogram, once it is performed, please ring Emma Stubbs on (03) 6226 4709 or free call 1800 638 124 to tell us when and where you had your mammogram.

The year I had my most recent mammogram was:

Year: _____

I had my most recent mammogram at:

☐ BreastScreen Location:

☐ Private Radiology Clinic Location:

My most recent mammogram is held by:

☐ Me
☐ BreastScreen
☐ Doctor/Clinic

Name of Doctor/Clinic:

Address of Doctor/Clinic:

Phone of doctor/clinic:
☐ I am not sure

Appendix 9: Follow-up 2 consent form, study invitation letter, information brochure and mammogram release form

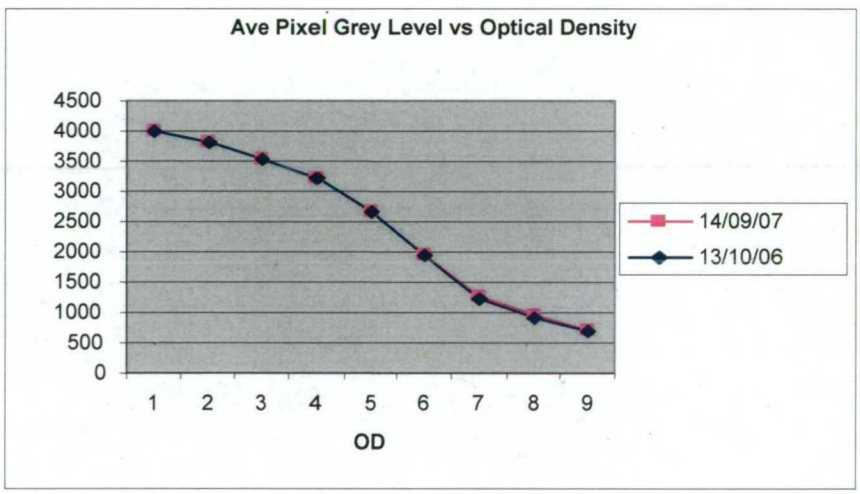
APPENDIX 10

Plot of optical density vs pixel grey level

Plot of optical density vs pixel grey level

A plot of the calibration measures (optical density and average pixel grey level) of the first mammographic images scanned (date 13/10/06) against the last batch of images scanned (date 14/09/07) is below in **Figure A10.1**.

Figure A10.1: Pixel grey level vs optical density (OD) for first and last set of mammogram film scans.

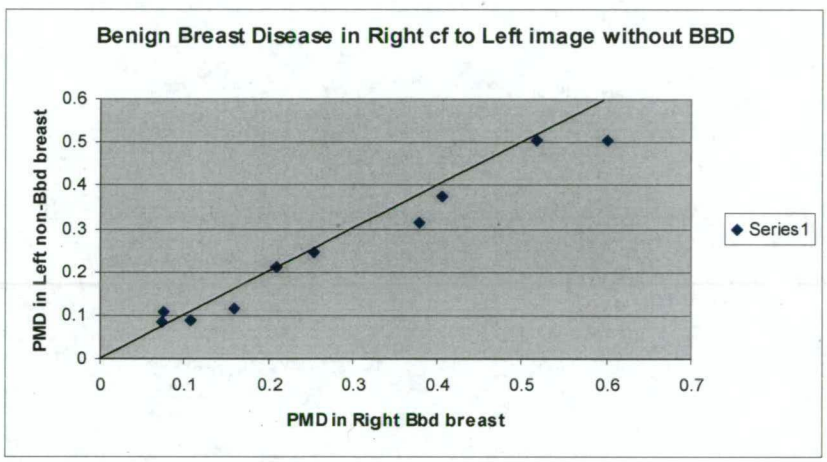


APPENDIX 11

Plot of right breast with benign breast disease vs left non-diseased breast

Percent mammographic density of the right breast with benign breast disease was plotted against the corresponding left non-diseased breast of the same woman (See **Figure A11.1**). A Spearman coefficient of 0.99 was calculated.

Figure A11.1: Percent mammographic density in the right benign breast diseased breast and the corresponding left non-diseased breast.



APPENDIX 12

Independent variables collected in follow-up 2

Independent variables collected and analysed in Chapter 7

- Age (years)
  - At interview
  - At mammogram
- Postmenopausal *
- Postmenopausal †
- Age at menarche (years)
- Number of livebirths
- Age at first livebirth (years)
- Ever breastfeed (%)
- Breastfeeding total mean (wks)
- Height (cm)
- Weight (kg)
- BMI (kg/m^2)
- EMH–final height (cm)
- Weight Change (kg)
  - 18 to 30 yrs
  - 18 yrs to current
  - 30 yrs to current
- Fertility drugs taken (y/n)
- Fertility cycles
- HRT
  - Ever used
  - Current use
  - Total use (years)
  - Type
- Hormonal Contraceptive
  - Ever used
  - Current use
  - Total use (years)
  - Age first used
- Ever used hormones for endometriosis
- Duration of hormone use to treat endometriosis (weeks)
- Ever used hormones for menstrual problems
- Ever used aspirin
- Ever used over the counter anti-inflammatories
- Ever used prescription anti-inflammatories)
- Smoking
  - Ever smoked
  - Currently smoke
- Alcohol use
  - Never or rarely drink
  - Occasionally (<once a week)
  - Once or twice a week
  - Three or more days a week
- Marital status
  - Married
  - De facto
  - Separated
  - Divorced
  - Widowed
  - Single
- Educational Level
  - Primary School
  - Intermediate/Year 11
  - High School/Year 11&12
  - Certificate/ Diploma
  - University Degree
  - Higher University Degree
- Country of Birth
  - Australia
  - UK
  - Other
- Benign Breast Disease
- Polycystic Ovary Syndrome
- Ovarian Cysts
- Uterine Fibroid
- Endometriosis[4]

Appendix 12: Independent variables collected in follow-up 2

Breast Cancer[5]
Vaginal/Uterine cancer
Breast cancer: 1st degree relative
Ovarian cancer: 1st degree relative

* Definition of postmenopausal: last period ≥ 52wks, and if HRT started before last period and current age was ≥55 years.
† Definition of postmenopausal: Same as above but women who had not had a period for ≥52 weeks because of hysterectomy (while retaining one or both ovaries), endometrial ablation, IUD, or hormone implants, they were considered to be premenopausal unless they were ≥55 years of age

Independent variables collected and analysed in Chapter 8

Birthweight (kg)
Birth-length (cm)
Bone age-chronological age (years).
Age bone age measurement (years)
Age at first assessment (years)
Height at first assessment (years)
Weight at first assessment (kg)
BMI at first assessment (kg/m^2)
Birthweight (kg)
Birth-length (cm)
Bone age-chronological age (years)
Age bone age measurement (years)
Weight change first year of treatment (kg)
BMI change first year of treatment (kg/m^2)
Age maximum height reached ≥15 years
Height change after 15 years (cm)

APPENDIX 13

Follow-up 2 CATI questionnaire

Menzies Research Institute

ID Number:_____
Interview Date:_____(dd/mm/yyyy)
Interviewer initials:_____
Time Start: _____
Time Finish:_____

Tall Girls Breast Density Study

QUESTIONNAIRE
(TO BE TRANSLATED TO CATI FORMAT)

My name is .....and I'm from the Menzies Research Institute, The University of Tasmania. X , the study administrator, contacted you earlier and arranged this time for you to be interviewed. Is this time still fine with you? It should take approximately X minutes.

Before we begin, I want to you to know that your answers to this interview are confidential and that your participation is voluntary. The questions I am about to ask relate to

1) Your current and past health, including an update of your reproductive history
2) Usage of some medications that may have an effect on the breast
3) Your lifestyle, and family history of breast and ovarian cancer; and
4) Your Mammogram history

When you participated in the earlier Tall Girls study you were interviewed on [date of interview]. A few of the questions that will be asked during this interview will repeat those asked in the earlier interview. This is so that we can update our information.

Please feel free to ask me to clarify or repeat a question anytime during the interview, and if you wish me to skip a question or stop this telephone call, please let me know.

If you would like to discuss any aspect of this interview with the study coordinator (Helen Jordan) or Chief Investigator (Alison Venn) please contact them during working hours on the numbers provided in your information brochure. If you do not have this available please feel free to ask me for these numbers following the interview.

Are you happy to continue? Do you have any questions before we start?

A. Medical and Surgical History

I'm going to ask questions about a number of illnesses and surgical procedures you may have had.

A1. Has a doctor ever told you that you had benign breast disease, such as a non-cancerous cyst or benign breast lump?

- Yes ☐
- No (go to A4) ☐
- Don't know (go to A4) ☐

A2. How old were you when this was first diagnosed?

Age of first diagnosis_____(years)

Don't Know ☐

A3 Which breast or breasts were affected?

- Both ☐
- Left only ☐
- Right only ☐

A4 Have you ever been diagnosed as having had an in-situ cancer of the breast ? (this is also known as a NON-malignant or NON-invasive breast cancer)

- Yes ☐
- No (go to A8) ☐

A5 Was it ductal carcinoma in-situ or lobular carcinoma in situ?

- Ductal ☐
- Lobular ☐
- Don't' Know ☐

A6 How old were you when this was first diagnosed?

Age _____ (years)

Don't Know ☐

A7 Which breast or breasts were affected?

Both ☐

Left only ☐

Right only ☐

A8 Have you ever been diagnosed as having had malignant or invasive breast cancer?

Yes ☐

No ☐ **(Go to A11 if said Yes to A4)**
(Go to A14 if said No to A4)

A9 How old were you when this was first diagnosed?

Age _____ (years)

Don't Know ☐

A10 Which breast or breasts were affected?

Both ☐

Left only ☐

Right only ☐

A11 *(If said yes to A4 or A8)* **Have you ever had surgery for the treatment of breast cancer?**

Yes ☐

No ☐ **(go to A14)**

A12 In which breast did you have surgery?

Both ☐

Left only ☐

Right only ☐

A13 What was your age when you first had this procedure?

Age_____ years

A14 Have you ever had any other form of breast surgery Or *(if no to A4 AND A8)* **Have you ever had breast surgery of any kind?**

Yes ☐

No **Go to A16** ☐

A15 What was the purpose of the surgery?

a) Removal of a breast lump ☐
A15_1 Age of procedure? _____**years**
A15_2 Which breast?

Both ☐

Left only ☐

Right only ☐

b) Breast Reduction ☐
A15_1 Age of procedure? _____**years**
A15_2 Which breast?

Both ☐

Left only ☐

Right only ☐

c) Breast Enlargement ☐

A15_1 Age of procedure? _____years

A15_2 Which breast?

Both ☐

Left only ☐

Right only ☐

d) Other ☐

Specify reason_____

A15_1 Age of procedure? _____years

A15_2 Which breast?

Both ☐

Left only ☐

Right only ☐

15a) Have you had any other type of breast surgery?

Yes (repeat Question 15)

No (go to A16)

A16 I have asked about breast cancer, has a doctor ever told you that you had any other type of cancer?

Yes ☐

No — (go to A18) ☐

A17. What was the type of cancer and your age when it was FIRST diagnosed?

First Cancer A17_1Type

Ovarian ☐

Vaginal/Uterine/Cervical ☐

Colon ☐

Other ☐ specify_____ *(If 'other' is skin cancer, ask if melanoma - only include if it is, or they don't know)*

A17_2 Age when first diagnosed _____ (years)

A17a: Has a doctor ever told you that you had any other form of cancer?

Yes ☐ *(Ask if it is a primary or secondary cancer - only say 'yes' if it is a primary cancer.)*

No ☐ **(Go to A18)**

A17_1 Second Cancer Type

Ovarian ☐

Vaginal/Uterine/Cervical ☐

Colon ☐

Other ☐ specify_____ *(If 'other' is skin cancer, ask if melanoma - only include if it is, or they don't know)*

A17a: Have you had any other form of cancer?

Yes ☐ *(Ask if it is a primary or secondary cancer - only say 'yes' if it is a primary cancer.)*

No ☐ **(Go to A18)**

A17_1 Third Cancer Type

Ovarian ☐

Vaginal/Uterine/Cervical ☐

Colon ☐

Other ☐ specify_____ *(If 'other' is skin cancer, ask if melanoma - only include if it is, or they don't know)*

A 18 Has a doctor ever told you that you had polycystic ovary syndrome?

Yes ☐

No ☐ (go to A20)

Don't Know ☐ (go to A20)

A 19 How old were you when polycystic ovary syndrome was first diagnosed?

Age_____(years)

Don't Know ☐

A 20 Has a doctor ever told you that you had cysts in one or both ovaries that was not diagnosed as Polycystic Ovary Syndrome?

Yes ☐

No ☐ **(go to A23)**

Don't know ☐ **(go to A23)**

A21 How old were you when ovarian cysts were first diagnosed?

Age_____(years)

A22 Have you ever had an ovarian cyst removed?

Yes ☐

No ☐

Don't Know ☐

A23 Have you ever had one or both ovaries removed?

Yes ☐

No ☐ **(go to A29)**

A24 How many ovaries have you had removed?

One ☐

Two ☐

A25 How old were you when you had your first ovary removed?

Years_____

Don't know ☐

A26 Why was it removed?

To treat ovarian cancer ☐

To prevent getting cancer in that ovary ☐

As part of treatment for breast cancer ☐

As part of prevention of breast cancer ☐

To help treat or stop endometriosis ☐

As part of a hysterectomy ☐

Other - specify reason ☐

Don't know ☐

(If participant had only one ovary removed when responding to A24, go to A29
If participant had two ovaries removed answer next question)

A27 How old were you when you had your second ovary removed?

Age _____Years

Don't know ☐

A28 Why was it removed?

To treat ovarian cancer	☐
To prevent getting cancer in that ovary	☐
As part of treatment for breast cancer	☐
As part of prevention of breast cancer	☐
To help treat or stop endometriosis	☐
As part of a hysterectomy	☐
Other - specify reason	☐
Don't know	☐

A29 Has a doctor ever diagnosed you as having had a uterine fibroid?

Yes ☐

No ☐ (go to A31)

DK ☐ (go to A31)

A30 How old were you when it was first diagnosed?

Age _____(years)

A31 Has a doctor ever diagnosed you as having had endometriosis?

Yes ☐

No ☐ (go to B1)

DK ☐ (go to B1)

A32 How old were you when it was first diagnosed?

Age _____(years)

A33 Was the endometriosis diagnosed as the result of a laparoscopy?

Yes ☐ (go to B1

No ☐ (go to A34)

Don't Know ☐ (go to A34)

A34 What diagnostic procedure was used to diagnose the endometriosis?

Laparotomy	☐
Hysteroscopy	☐
Hysterectomy	☐
Ultrasound (and/or MRI)	☐
Other surgical procedure, specify	☐
Don't Know	☐

B. Height and Weight

Now I'm going to ask about your height and weight

B1. How tall are you without shoes on? Please be as accurate as you can. (Give fraction of an inch or centimetre)

e.g. 5 feet 11 inches 1/4 OR 181.5 cm

Feet _____ Inches _____ ☐ OR Centimetres____

Don't know

B2. What is your current weight without clothes?

stone _____ pounds_____ OR kilograms _____

Don't know

B3. What was your weight when you were between 18 and 21 years old?

stone ____pounds____ OR kilograms ____
Don't know

B4 What was your weight at 30 years?

stone ____pounds____ OR kilograms ____
Don't know

C. Reproductive History

Now I'd like to ask about your pregnancies and use of fertility drugs.

Pregnancy History

C1 Since your last interview on [date of interview], have you been pregnant? (Including all your pregnancies: miscarriages, stillbirths, terminations, molar or tubal pregnancies, as well as live births or current pregnancy)

Yes ☐

No (go to C9) ☐

C2 How many times have you been pregnant since [date of last interview]?

____Number of times pregnant

C3 For the first / next of these pregnancies since [date of last interview/DOI] what month and year did this pregnancy end?

1st pregnancy:

☐☐/☐☐☐☐ Month/Year (Go to C4)

Currently pregnant ☐ **How many months pregnant? ____ (Go to C9)**

2nd pregnancy:

☐☐/☐☐☐☐ Month/Year (Go to C4)

Currently pregnant ☐ **How many months pregnant? ____ (Go to C9)**

3rd pregnancy:

☐☐/☐☐☐☐ Month/Year (Go to C4)

Currently pregnant ☐ **How many months pregnant? ____ (Go to C9)**

4th pregnancy:

☐☐/☐☐☐☐ Month/Year (Go to C4)

Currently pregnant ☐ **How many months pregnant? ____ (Go to C9)**

C4 A full term pregnancy is 40 weeks. How many weeks pregnant were you when this pregnancy ended?

First pregnancy since DOI
_____Weeks (go to C5)

Second pregnancy since DOI
_____Weeks (go to C5)

Third pregnancy since DOI
_____Weeks (go to C5)

Fourth pregnancy since DOI
_____Weeks (go to C5)

Fifth pregnancy since DOI
_____Weeks (go to C5)

C5 How did this pregnancy end?

First Pregnancy:

- Live birth ☐ **C5a Specify** single birth ☐ **Go to C6**
  twin ☐ **Go to C6**
  other multiple birth ☐ **Go to C6**
- Stillbirth ☐ **Return to C3 if more pregnancies or to C9 if not**
- Miscarriage ☐ **Return to C3 if more pregnancies or to C9 if not**
- Termination ☐ **Return to C3 if more pregnancies or to C9 if not**
- Ectopic ☐ **Return to C3 if more pregnancies or to C9 if not**
- Molar Pregnancy ☐ **Return to C3 if more pregnancies or to C9 if not**
- Don't Know ☐ **Return to C3 if more pregnancies or to C9 if not**

Second Pregnancy:

- Live birth ☐ **C5a Specify** single birth ☐ **Go to C6**
  twin ☐ **Go to C6**
  other multiple birth ☐ **Go to C6**
- Stillbirth ☐ **Return to C3 if more pregnancies or to C9 if not**
- Miscarriage ☐ **Return to C3 if more pregnancies or to C9 if not**
- Termination ☐ **Return to C3 if more pregnancies or to C9 if not**
- Ectopic ☐ **Return to C3 if more pregnancies or to C9 if not**
- Molar Pregnancy ☐ **Return to C3 if more pregnancies or to C9 if not**
- Don't Know ☐ **Return to C3 if more pregnancies or to C9 if not**

Third Pregnancy:

- Live birth ☐ **C5a Specify** single birth ☐ **Go to C6**
  twin ☐ **Go to C6**
  other multiple birth ☐ **Go to C6**
- Stillbirth ☐ **Return to C3 if more pregnancies or to C9 if not**

- Miscarriage ☐ **Return to C3 if more pregnancies or to C9 if not**
- Termination ☐ **Return to C3 if more pregnancies or to C9 if not**
- Ectopic ☐ **Return to C3 if more pregnancies or to C9 if not**
- Molar Pregnancy ☐ **Return to C3 if more pregnancies or to C9 if not**
- Don't Know ☐ **Return to C3 if more pregnancies or to C9 if not**

Fourth Pregnancy:

- Live birth ☐ **C5a Specify** single birth ☐ **Go to C6**
  twin ☐ **Go to C6**
  other multiple birth ☐ **Go to C6**
- Stillbirth ☐ **Return to C3 if more pregnancies or to C9 if not**
- Miscarriage ☐ **Return to C3 if more pregnancies or to C9 if not**
- Termination ☐ **Return to C3 if more pregnancies or to C9 if not**
- Ectopic ☐ **Return to C3 if more pregnancies or to C9 if not**
- Molar Pregnancy ☐ **Return to C3 if more pregnancies or to C9 if not**
- Don't Know ☐ **Return to C3 if more pregnancies or to C9 if not**

Fifth Pregnancy:

- Live birth ☐ **C5a Specify** single birth ☐ **Go to C6**
  twin ☐ **Go to C6**
  other multiple birth ☐ **Go to C6**
- Stillbirth ☐ **Return to C3 if more pregnancies or to C9 if not**
- Miscarriage ☐ **Return to C3 if more pregnancies or to C9 if not**
- Termination ☐ **Return to C3 if more pregnancies or to C9 if not**
- Ectopic ☐ **Return to C3 if more pregnancies or to C9 if not**
- Molar Pregnancy ☐ **Return to C3 if more pregnancies or to C9 if not**
- Don't Know ☐ **Return to C3 if more pregnancies or to C9 if not**

C6. (*if said 'livebirth' to* C5) **Did you commence breastfeeding after this birth?**

First pregnancy resulting in live birth since DOI

Yes ☐ **(If there were no more pregnancies go to C7, If more go to C8a)**

No ☐ **(Return to C3 if more pregnancies or go to C9 if not)**

Second pregnancy resulting in live birth since DOI

Yes ☐ **(If there were no more pregnancies go to C7, If more go to C8a)**

No ☐ **(Return to C3 if more pregnancies or go to C9 if not)**

Third pregnancy resulting in live birth since DOI

Yes ☐ **(If there were no more pregnancies go to C7, If more go to C8a)**

No ☐ **(Return to C3 if more pregnancies or go to C9 if not)**

Fourth pregnancy resulting in live birth since DOI

Yes ☐ **(If there were no more pregnancies go to C7, If more go to C8a)**

No ☐ **(Return to C3 if more pregnancies or go to C9 if not)**

C7 Are you currently breastfeeding?

No ☐ **Go to C8a**

Yes ☐ **Go to C8b**

C8a How long did you breastfeed?

_____Days (go to C3)
_____Weeks (go to C3)
_____Months (go to C3)

C8b How long have you breastfed following this pregnancy so far?

_____Days
_____Weeks
_____Months

<u>Infertility Drugs:</u>

C9 Have you ever seen a doctor because you were having trouble getting pregnant?

Yes ☐

No (go to Section D) ☐

C10 Have you ever taken fertility drugs for the treatment of infertility?

Yes ☐

No (go to Section D) ☐

C11 In total, what is the number of cycles of fertility drug treatment that you've had (include IVF cycles and ovulation induction)?

_____cycles of fertility treatment

C12 How old were you when you first started fertility drug treatment?

Age _____ (Years)

C13 How old were you when you last had fertility drug treatment?

Age _____ (Years) *(give current age if still using treatment)*

Section D. Menopause and Hormone Replacement Therapy

Now I'm going to ask questions about menopause and hormone replacement therapy or HRT

D1. How long ago was your last period?

_____Days
_____Weeks
_____Months
_____Year(s)

If less than one year go to D4
If one year or more continue to D2.

D2 What age were you when you had your last period?

Age _____(years)

D3. Why did your menstrual periods stop?
(Read options & record only one answer)

Natural menopause (that is, periods stopped by themselves) ☐
Hysterectomy (uterus or womb removed) ☐
Both ovaries removed ☐
Radiation or chemotherapy ☐
Pregnant/breast feeding ☐
Serious illness (e.g. Anorexia) ☐
Strenuous exercise ☐

Other (specify) _____
Don't know ☐

D4. Have you ever taken prescription oestrogens, progesterone or other female hormones for menopause, (that is, prescription hormone replacement therapy or HRT)?

The preparation may be pills, injections, skin patches. This question does not include birth control pills or hormonal contraceptives.

Yes ☐
No ☐ **(go to D17)**
Don't know ☐ **(go to D17)**

D5. Were you still having periods when you first took HRT?

Yes ☐**(go to D7)**
No ☐
Don't know ☐**(go to D7)**

D6. How long after your last period did you take HRT?

_____Months
_____Weeks
_____Years
Don't know ☐

D7 How old were you when you first used/resumed/changed the HRT medication?

1_____ Age (years) **(go to D8)**
2_____ Age (years) **(go to D8)**
3_____ Age (years) **(go to D8)**
4_____ Age (years) **(go to D8)**
5_____ Age (years) **(go to D8)**

D8 What was the name of the hormone medication you took (when you first started HRT / at this time)?

1.Name of Medication_____ **(go to D10)**

Don't Know **(continue to D9)** ☐

2. Name of Medication_____ **(go to D10)**

Don't Know **(continue to D9)** ☐

3. Name of Medication_____ **(go to D10)**

Don't Know **(continue to D9)** ☐

4. Name of Medication_____ **(go to D10)**

Don't Know **(continue to D9)** ☐

D9 Were the hormones in the medication :

1. Progesterone Only ☐
   Progesterone and oestrogen ☐
   Oestrogen only ☐
   DK ☐
   Go to D10

2. Progesterone Only ☐
   Progesterone and oestrogen ☐
   Oestrogen only ☐
   DK ☐
   Go to D10

3. Progesterone Only ☐
   Progesterone and oestrogen ☐
   Oestrogen only ☐
   DK ☐
   Go to D10

4. Progesterone Only ☐
   Progesterone and oestrogen ☐
   Oestrogen only ☐
   DK ☐
   Go to D10

D10 How was it taken?

1. Orally ☐
   Implants ☐
   Injections ☐
   Vaginally ☐
   Other ☐
   Go to D11

2. Orally ☐
   Implants ☐
   Injections ☐
   Vaginally ☐
   Other ☐
   Go to D11

3. Orally ☐
   Implants ☐
   Injections ☐
   Vaginally ☐
   Other ☐
   Go to D11

4. Orally ☐
   Implants ☐
   Injections ☐
   Vaginally ☐
   Other ☐

Go to D11

D11 At any time after this, did you stop taking HRT for more than 12 months, or change the medication you were using?

1. Yes ☐ **Go to D12**
   No ☐ **Go to D16**
2. Yes ☐ **Go to D12**
   No ☐ **Go to D16**
3. Yes ☐ **Go to D12**
   No ☐ **Go to D16**
4. Yes ☐ **Go to D12**
   No ☐ **Go to D16**

D12 For how many months did you use it before you stopped or changed it at this time?

1. _____months

   Go to 13
2. _____months

   Go to 13
3. _____months

   Go to 13
4. _____months

   Go to 13

D13 Did you stop or change it?

1. Stopped (go to D14) ☐
   Changed (go back to D7 and repeat cycle) ☐
2. Stopped (go to D14) ☐
   Changed (go back to D7 and repeat cycle) ☐
3. Stopped (go to D14) ☐
   Changed (go back to D7 and repeat cycle) ☐
4. Stopped (go to D14) ☐
   Changed (go back to D7 and repeat cycle) ☐

D14 How old were you when you stopped it?

1_____ Age (years) (go to D15)

2_____ Age (years) (go to D15)

3_____ Age (years) (go to D15)

4_____ Age (years) (go to D15)

5_____ Age (years) (go to D15)

D15 Did you ever take HRT again after that?

1.	Yes	(go back to D7)	☐
	No	(go to D17)	☐
2.	Yes	(go back to D7)	☐
	No	(go to D17)	☐
3.	Yes	(go back to D7)	☐
	No	(go to D17)	☐
4.	Yes	(go back to D7)	☐
	No	(go to D17)	☐

D16 Are you currently using HRT?

Yes (go to D17)
No (go to D16_1)

D16_1 How old were you when you last took HRT?

Age____years

D17 Have you ever taken tamoxifen, raloxifene or other anti-oestrogen medication? (such as Evista, Tamoxen, Genox, Nolvadex, Noxiton, Tamosin)

Yes	☐
No (go to Section E)	☐
Don't know (go to Section E)	☐

D18 Which anti-oestrogen did you take?

Tamoxifen ☐
Raloxifene ☐
Other, specify_____

Don't Know ☐

D19 How old were you when you first took the medication?

____Age (years)

D20 Are you currently taking Tamoxifen, Raloxifene or other anti-oestrogen medication?

Yes (Go to D22)	☐
No	☐

D21. How old were you when you last took Tamoxifen, Raloxifene or other anti-oestrogen medication?

Age _____Years

D22. In total for how many weeks, months or years have you taken them?

_____Weeks
_____Months
_____Years

Section E. Contraceptive Use

Now I am going to ask you about your use of hormonal contraception.

E1. Have you ever used birth control pills or other hormonal contraceptives such as implants or injections? Please include contraceptives used for other reasons than contraception.

Yes ☐

No ☐ **(go to E11)**

Don't know ☐ **(go to E11)**

E2 Were these contraceptive hormones used for anything other than contraception?

Yes ☐ Specify reason/condition_____

No ☐

E3 How old were you when you first/next used the pill or hormonal contraceptive?

1. ____Age (years)
2. ____Age (years)
3. ____Age (years)
4. ____Age (years)
5. ____Age (years)
6. ____Age (years)

E4 How was it taken?

1.	orally	☐	
	implants	☐	
	injections	☐	
	other	☐	Specify _____
2.	orally	☐	
	implants	☐	
	injections	☐	
	other	☐	Specify _____
3.	orally	☐	
	implants	☐	
	injections	☐	
	other	☐	Specify _____
4.	orally	☐	
	implants	☐	
	injections	☐	
	other	☐	Specify _____
5.	orally	☐	
	implants	☐	
	injections	☐	
	other	☐	Specify _____
6.	orally	☐	
	implants	☐	
	injections	☐	
	other	☐	Specify _____

7. orally ☐
   implants ☐
   injections ☐
   other ☐ Specify _____

E5 At any time after this, did you ever stop using it for 12 months or more?

1. Yes ☐
   No (go to E9) ☐
2. Yes ☐
   No (go to E9) ☐
3. Yes ☐
   No (go to E9) ☐
4. Yes ☐
   No (go to E9) ☐
5. Yes ☐
   No (go to E9) ☐
6. Yes ☐
   No (go to E9) ☐
7. Yes ☐
   No (go to E9) ☐

E6 How old were you then?

1. ____Age (years)
2. ____Age (years)
3. ____Age (years)
4. ____Age (years)
5. ____Age (years)
6. ____Age (years)

E7 For how many weeks, months or years did you take it before you first stopped/stopped it this time?

1. _____Weeks
   _____Months
   _____Years
   (go to E8)
2. _____Weeks
   _____Months
   _____Years
   (go to E8)
3. _____Weeks
   _____Months
   _____Years
   (go to E8)
4. _____Weeks
   _____Months
   _____Years
   (go to E8)

5. _____Weeks

_____Months

_____Years

(go to E8)

6. _____Weeks

_____Months

_____Years

(go to E8)

E8 Did you ever take the pill or hormonal contraceptive again after that?

1. Yes (Go back to E3 and repeat cycle) ☐
   No (go to E11) ☐
2. Yes(Go back to E3 and repeat cycle) ☐
   No (go to E11) ☐
3. Yes (Go back to E3 and repeat) ☐
   No (go to E11) ☐
4. Yes (Go back to E3) ☐
   No (go to E11) ☐
5. Yes (Go back to E3) ☐
   No (go to E11) ☐
6. Yes (Go back to E3) ☐
   No (go to E11) ☐

E9 Are you currently taking the pill or a hormonal contraceptive?

Yes ☐(go to E11)

No ☐

E10 How old were you when you last took the pill or a hormonal contraceptive?

_____Age (years)

E11 Apart from the oral contraceptive pill or hormonal contraceptives for which we have covered, have you taken any other hormonal medication for ?

E11_1 *(if said yes to A33)* Endometriosis

Yes ☐ No ☐ Go to E11_2

a) If yes, What is the name of the hormone medication you were taking for this condition?

Name of medicine_____

b) In total for how many weeks, months or years have you taken hormonal medication for this condition? (If you took this medication over different time periods, add up together all the times you took medications).

Weeks
Months
Years

c) How old were you when you first took this medication?

Age____(years)

d) How old were you when you last took this medication?

Age____(years)

E11_2 *(if said yes to A20)* **Polycystic Ovary Syndrome**

Yes ☐

No ☐ Go to E11_3

a) If yes, What is the name of the hormone medication you were taking for this condition?

Name of medicine_____

b) In total for how many weeks, months or years have you taken hormonal medication for this condition? (If you took this medication over different time periods, add up together all the time you took medications).

Weeks
Months
Years

c) How old were you when you first took this medication?

Age_____(years)

d) How old were you when you last took this medication?

Age_____(years)

E11_3 *(if said yes to A22)* **Ovarian Cysts (other than PCO)**

Yes ☐ No ☐ Go to E11_4

a) If yes, What is the name of the hormone medication you were taking for this condition?

Name of medicine_____

b) In total for how many weeks, months or years have you taken hormonal medication for this condition? (If you took this medication over different time periods, add up together all the time you took medications).

Weeks
Months
Years

c) How old were you when you first took this medication?

Age_____(years)

d) How old were you when you last took this medication?

Age_____(years)

E11_4 *(if said yes to A29)* **Uterine Fibroid**

Yes ☐ No ☐ Go to E11_5

a) If yes, What is the name of the hormone medication you were taking for this condition?

Name of medicine_____

b) In total for how many weeks, months or years have you taken hormonal medication for this condition? (If you took this medication over different time periods, add up together all the time you took medications).

Weeks
Months
Years

c) How old were you when you first took this medication?

Age_____(years)

d) How old were you when you last took this medication?

Age_____(years)

E11_5 Menstrual Problems not due to endometriosis, ovarian cysts, PCO or uterine fibroids.

Yes ☐ No ☐ Go to E12

If yes, What is the name of the hormone medication you were taking for this condition?

Name of medicine_____

In total for how many weeks, months or years have you taken hormonal medication for this condition? (if you took this medication over different time periods, add up together all the time you took medications).

_____Weeks
_____Months
_____Years

c) How old were you when you first took this medication?

Age_____(years)

d) How old were you when you last took this medication?

Age_____(years)

E12 Have you been given hormone medication for anything else as an adult?

Yes ☐

No (go to F1) ☐

E13 For which conditions?

1. Conditon_____
2. Condition_____
3. Conditon _____

E14 (For each of the conditions in E13) **What hormonal medication was used for *the condition*?**

1. Medication for Condition 1_____
2. Medication for Condition 2_____
3. Medication for Condition 3_____

E15 (For each of the conditions in E13) **In total for how many weeks, months or years have you taken hormonal medication for this condition?** (if you took this medication over different time periods, add up together all the time you took medications)

Condition 1
_____Weeks
_____Months
_____Years

Condition 2
_____Weeks
_____Months
_____Years

Condition 3
_____Weeks
_____Months
_____Years

E16 What age were you when you last used this medication?

Age_____(Years)

<u>Anti-inflammatories</u>

I am now going to ask about your use of anti-inflammatories

In table format:

F1 Have you ever taken Aspirin (*such as Aspro, Disprin, Cardiprin, Cartia , Solprin*) at least twice a week for a month or longer?

Yes ☐

No ☐(go to F5)

Don't Know ☐(go to F5)

F2 How many times per day or week did you take this medication, when you were taking it at least twice a week for a month or longer?

_____Per Day
_____Per Week

F3 For how many months or years, in total, have you taken this medication for at least twice a week for a month or longer?

_____Months
_____Years

F4 Was it standard dose or low dose Aspirin that you took when taking it at least twice a week for a month or longer? (e.g. Cartia, Cardiprin)

Standard Dose ☐
Low Dose ☐
Both ☐
DK ☐

F5 Have you ever taken over the counter anti-inflammatories at least twice a week for a month or longer?

Yes ☐
No ☐(go to F9)
Don't Know ☐(go to F9)

F6 How many times per day or week did you take over the counter anti-inflammatories, when you were taking it at least twice a week for a month or longer?

_____Per Day
_____Per Week

F7 For how many years or months, in total, have you taken this over the counter anti-inflammatories for at least twice a week for a month or longer?

_____Months
_____Years

F8 What was the name of the medication or medications?

_____medication

_____medication

_____medication

F9 Have you ever taken anti-inflammatories that require a prescription by a doctor at least twice a week for a month or longer?

Yes ☐
No ☐(go to Section G)
Don't Know (go to Section G) ☐

F10 What was the name of the medication or medications? (Give some examples in box if needed - prompt for Vioxx, Celebrex or Mobic)

_____medication

_____medication

Don't Know ☐

F11 How many times per day or week did you take anti-inflammatories that require a prescription by a doctor, when you were taking it at least twice a week for a month or longer?

_____Per Day
_____Per Week

F12 For how many years or months, in total, have you taken anti-inflammatories that require a prescription by a doctor at least twice a week for a month or longer?

_____Months
_____Years

G. Smoking

The next questions are about your history of cigarette smoking.

G1 Which of the following best describes your smoking status now?

I have never smoked	(go to Section H)	☐
I used to smoke occasionally	(go to G2)	☐
I used to smoke regularly	(go to G2)	☐
I now smoke occasionally	(go to G4)	☐
I now smoke regularly	(go to G4)	☐

G2 If you used to smoke, what age were you when you last smoked?

Age_____(years)

G3 If you used to smoke, how many cigarettes did you usually smoke in a day?

cigarettes per day_____

Go to G5

G4 How many cigarettes do you usually smoke in a day?

cigarettes per day_____

G5 Have you ever smoked daily for six months or more?

Yes ☐

No ☐

G6 At what age did you start smoking?

Age_____(years)

H. Alcohol

The next questions are about your alcohol consumption patterns.

H1 Which of these statements best describes how often you usually drink alcohol (such as beer, wine or spirits)? (read responses)

I never drink alcohol (go to Section J)	☐
I drink rarely	☐
Occasionally, but less than once a week	☐
On 1 or 2 days a week	☐
On 3 or 4 days a week	☐
On 5 or 6 days a week	☐
Every day	☐

A "standard drink" is a small glass of wine or middy of beer, a nip of spirits, or a mixed drink.

H2 On a day when you drink alcohol, how many drinks do you usually have?

_____drinks

H3 Have you ever consumed alcohol daily for six months or more?

Yes ☐

No ☐

J. FAMILY HISTORY

These questions are about breast and ovarian cancer in your family; I'm going to ask about your biological relatives, not including step or adoptive relatives.

J1. Has your mother ever had breast cancer?

No ☐

Yes, ☐ J1a.Age 1st diagnosed? _____years old

Don't know ☐

J2. Do you have any sisters who have had breast cancer?

No ☐

Yes, ☐ J2a If yes, how many sisters? _____

J2b If yes, age first diagnosed

1ST Sister _____yrs
2ND Sister _____yrs
3RD Sister yrs _____
4TH Sister _____yrs

Don't Know ☐

Don't know ☐

J3 Do you have any daughters who have had breast cancer?

No ☐

Yes ☐ J3a If yes, how many daughters? _____

J3b If yes, age first diagnosed:

1st daughter _____ yrs
2nd daughter _____yrs
3rd daughter _____yrs
4th daughter _____yrs

Don't Know ☐

Don't know ☐

J4. Are there any aunts on your father's side who have had breast cancer?

No ☐

Yes ☐ J4a If yes, how many aunts? _____

J4b If yes, age first diagnosed

1ST Aunt _____yrs
2ND Aunt _____yrs
3RD Aunt _____yrs
4TH aunt _____yrs

Don't Know ☐

Don't know ☐

J5 Are there any aunts on your mother's side who have had breast cancer?

No ☐

Yes ☐ J5a If yes, how many aunts? _____

J5b If yes, age first diagnosed

1ST Aunt _____yrs
2ND Aunt _____yrs
3RD Aunt _____yrs
4TH aunt _____yrs
Don't Know

Don't know ☐

J6. Has either of your grandmothers had breast cancer?

No ☐ Go to J9

Maternal ☐ Go to J7

Paternal ☐ Go to J8

Both ☐ Go to J7 and J8

Don't Know ☐ Go to J9

J7 What age did the maternal grandmother have breast cancer?

Age

Don't know ☐

J8 What age did the paternal grandmother have breast cancer?

Age

Don't know ☐

J9. Have any of your sisters, or mother, or daughters had ovarian cancer?

No ☐ Go to J11

Yes ☐ Go to J10

Don't Know ☐ Go to J11

J10 How many of your sisters, or aunts, or daughters have had ovarian cancer?

_____Number of sisters, mother or daughters

J11 Are there any aunts on your mother's side who have had ovarian cancer?

No ☐ Go to J13

Yes ☐ Go to J12

Don't Know ☐ Go to J12

J12 What is the number of aunts on mother's side who have had ovarian cancer?

Number of Aunts on mother's side_____

J13 Are there any aunts on your father's side who have had ovarian cancer?

No ☐ Go to J15

Yes ☐ Go to J14

J14 What is the number of aunts on your father's side who have had ovarian cancer ?

Number of Aunts on mother's side_____

J15 Has any of your grandmothers had ovarian cancer?

Fathers side ☐

Mothers Side ☐

Both ☐

No ☐

Don't Know ☐

K. Background Information

I'm going to finish off by asking some questions about your background

Can you give me your date of birth so we can confirm it with earlier records?

K1. Date of birth? ___ /____ /____(dd/mm/yyyy)

K2. What is the highest level of education you have completed? (one response)

Primary school (some or all) ☐

Intermediate certificate/year 10 (or equivalent) ☐

Higher School &/or Leaving Certificate/Year 11 & 12 ☐

Trade/apprenticeship (e.g. Chef, Hairdresser) ☐

Certificate/Diploma (e.g. Child Care, Technician) ☐

University degree ☐

Higher University degree (e.g. Grad Dip, Masters, PhD) ☐

K3. What is your present marital status? (one response only)

- Married ☐
- De facto ☐
- Separated ☐
- Divorced ☐
- Widowed ☐
- Single ☐

K4. In which country were you born?

K4a You

What about
- K4b Your Mother
- K4c Your Father
- K4d Your mother' mother
- K4e Your mother's father
- K4f Your father's mother
- K4g Your father's father

APPENDIX 14

Ethical approvals obtained for follow-up 2

Tasmania
DEPARTMENT of HEALTH and HUMAN SERVICES

UNIVERSITY OF TASMANIA

HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

11 August 2005

A/Prof Alison Venn
Menzies Centre
Private Bag 23

Dear A/Prof Venn

REF NO: H0008334
TITLE: Adolescent exposure to hormone treatment for tall stature in girls: long terms effects on breast tissue

The Southern Tasmania Health and Medical Human Research Ethics Committee considered and approved the above documentation at its meeting on **21 July 2005.**

All committees operating under the Human Research Ethics Committee (Tasmania) Network are registered and required to comply with the *National Statement on the Ethical Conduct in Research Involving Humans 1999* (NHMRC guidelines).

Therefore, the Chief Investigator's responsibility is to ensure that:

(1) The individual researcher's protocol complies with the HREC approved protocol.

(2) Modifications to the protocol do not proceed until approval is obtained in writing from the HREC.

(3) The confidentiality and anonymity of all research subjects is maintained at all times, except as required by law.

(4) Clause 2.37 of the *National Statement* states:

An HREC shall, as a condition of approval of each protocol, require that researchers immediately report anything which might warrant review of ethical approval of the protocol, including:

a. Serious or unexpected adverse effects on participants;

b. Proposed changes in the protocol; and

c. Unforeseen events that might affect continued ethical acceptability of the project.

The appropriate forms for reporting such events in relation to drug trials can be located at the website below. All adverse events must be reported regardless of whether or not the event, in your opinion, is a direct effect of the drug being tested.
http://www.research.utas.edu.au/rdo/ethics/human.htm

(5) All subjects must be provided with the current Patient Information Sheet and Consent Form as approved by the Ethics committee.

(6) The Committee is notified if any investigators are added to, or cease involvement with, the project.

Research and Development Office, University of Tasmania, Private Bag 01 HOBART TAS 7001
Tel: (03) 62262763 Fax: (03) 62262765 Email: Amanda.Mcaulty@utas.edu.au

(7) This study has approval for 4 years contingent upon annual review. An *Progress Report* is to be provided on the anniversary date of your approval. Your first report is due 21 July 2006. You will be sent a courtesy reminder closer to this due date.

Clause 2.35 of the National Statement states:

As a minimum an HREC must require at regular periods, at least annually, reports from principal researchers on matters including:

a. *progress to date or outcome in the case of completed research;*
b. *maintenance and security of records;*
c. *compliance with the approved protocol; and*
d. *compliance with any conditions of approval*

(8) A *Final Report* and a copy of the published material, either in full or abstract, must be provided at the end of the project.

Should you have any queries please do not hesitate to contact Rachael Cowen Kitanovic on 62261751 in the first instance.

Yours sincerely

RCkitanovic

Amanda McAuliy
Executive Officer
Human Research Ethics Committee (Tasmania) Network

ACT HEALTH HUMAN RESEARCH ETHICS COMMITTEE

Outcome of Consideration of Protocol

Submission No: ETH.5/06.313 **Date of Approval:** 14 August 2006

Project Title:
Exposure to High Dose Estrogens in Adolescence: Long Term Effects on Mammographic Breast Density

Submitted by:
Associate Professor Alison Venn

Your project was considered by the ACT Health Human Research Ethics Committee and approved for a period of one year

Further Action required:

Review due: August 2007

The Ethics Committee require as part of the review process that:

- At regular periods, and not less frequently than annually, Principal Investigators are to provide reports on matters including:
  - security of records
  - compliance with approved consent procedures and documentation
  - compliance with other approved procedures.
  - as a condition of approval of the protocol, that Investigators report immediately:
  - adverse affects on subjects
  - proposed changes in the protocol
  - unforeseen events that might affect continued ethical acceptability of the project.
- All published reports to carry an acknowledgement stating:
  - approved on 14 August 2006 by the ACT Health and Community Care Human Research Ethics Committee.

MS ELIZABETH GRANT AM, CHAIR Date: 14 August 2006

Government of South Australia
Department of Health

Human Research Ethics Committee

ABN 97 643 356 590
Citi Centre Building
11 Hindmarsh Square
Adelaide SA 5000

PO Box 287
Rundle Mall
Adelaide SA 5000

Ms Helen Jordan
6 Casley Street
BENDIGO VIC 3550

Dear Ms. Jordan,

RE: Exposure to high dose estrogens in adolescence: long term effects on mammographic breast density.

Thank you submitting the above project to the Department of Health Human Research Ethics Committee for consideration. The Committee met on the 14th December 2005 to review your proposal.

I am pleased to inform you that ethics approval has been given to the above proposal.

Approval is given subject to:

- The plain language statement containing further specific information relating to the risks (both medical and psycho-social) associated with participation in this project, to ensure that the participants can make an informed decision about their involvement.
- The research being conducted in accordance with the 'National Statement on Ethical Conduct in Research Involving Humans.'
- Provision of a final report when the project is completed.
- Immediate notification to HREC of any adverse events involving participants.
- Immediate notification of any unforeseen events that might affect continued ethical acceptability of the project.
- Submission of any significant changes to the original proposal. Such changes should be approved by the HREC before they are implemented.
- Immediate advice, giving reasons, if the project is discontinued before its completion.

Approval is given for a period of three (3) years only, and if the research is more prolonged than this, a new submission will be required.

Should you have any questions or concerns, please contact Sarah Lawson, Executive Officer of the HREC, Tel 8226 6367 or sarah.lawson@health.sa.gov.au.

We wish you all the best with the *"Exposure to high dose estrogens in adolescence: long term effects on mammographic breast density."* project.

Yours sincerely,

Ian Olver
A/CHAIRPERSON
HUMAN RESEARCH ETHICS COMMITTEE

/12/2005

cancerinstitute

Date: 4th August 2006

Cancer Institute NSW
Level 1, Biomedical Building
Australian Technology Park
Eveleigh NSW 2015

PO Box 41, Alexandria NSW 1435

T 02 8374 5600
F 02 8374 5700
www.cancerinstitute.org.au

ABN 48 538 442 594

Associate Professor Alison Venn
Deputy Director
Menzies Research Institute
University of Tasmania,
Private Bag 23
Hobart, TAS 7001

Dear Associate Professor Venn,

Re: Cancer Institute NSW Reference Number: 2006/06/003

Exposure to high dose estrogen in adolescence: long term effects on mammographic breast density

Thank-you for your correspondence dated 13th July 2006, were you responded to the Cancer Institute NSW Ethics Committee's letter of 20th June 2006.

I am pleased to inform you that following a review of your letter, formal ethical approval for your study has been given. The Committee wishes to reconfirm, that as of June 5th 2004, the Cancer Institute NSW Ethics Committee is the primary ethics committee responsible for all ongoing monitoring of these approvals and that ongoing approval is conditional on the following:

- That the study continues to be conducted in accordance with the approved application and all subsequent amendments.
- Compliance with the NHMRC *National Statement on Ethical Conduct in Research Involving Humans 1999*, NSW Health Privacy Manual (Version 1, 2004) and the *Health Records & Information Privacy Act (NSW) 2002*.
- An Annual Report is submitted, no later than August 2007, and at the study completion.
- The Institute is to be provided with final publications or reports generated by the study.
- The Cancer Institute NSW Ethics Committee is to be advised of any intended protocol amendments unforseen and/or adverse events occurring in relation to the study.
- Compliance with policies and guidelines issued by the Cancer Institute NSW Ethics Committee.

Should you require any further assistance in relation to this study, please feel free to contact me on (02) 8374-5759 or kirsten.legione@cancerinstitute.org.au

Yours sincerely,

Kirsten Legione
Project Officer – Ethics

7 November 2005

BreastScreen
VICTORIA

Coordination Unit

31 Pelham Street
Carlton South
Victoria 3053

Phone 03 9660 6888
Facsimile 03 9662 3881

Email info@breastscreen.org.au
Website www.breastscreen.org.au

Associate Professor Alison Venn
C/- Ms Helen Jordan
Program Evaluation Unit
School of Population Health
The University of Melbourne
4th Floor, 207 Bouverie Street
Carlton Vic 3010

Email: h.jordan@unimelb.edu.au

Dear Alison

Re: Exposure to high dose estrogens in adolescence: long term effects on mammographic breast density

The above project was considered by the BreastScreen Victoria Research and Evaluation Committee ("REC") on 25 October 2005.

I refer to your intention to ask women who have not had a mammogram in the last two years to have one at a convenient BreastScreen service. The REC believed many of the women in your study would be in the 40 to 50 age group. It was recommended that a statement about the smaller benefit of mammography for this age group should be provided to these women.

Subject to the above, the REC agreed that your project be endorsed.

We look forward to receiving a summary of the project findings at completion.

Yours sincerely

Anna Maloney
Project Officer
Quality and Research in Practice
BreastScreen Victoria
p: (03) 9660 6857
e: amaloney@breastscreen.org.au

BreastScreen Victoria Inc.
Regd. no. A0025878W

ABN 54 505 206 361

Queensland Government

Queensland Health

CANCER SCREENING SERVICES UNIT
PUBLIC HEALTH SERVICES BRANCH

Enquiries to: Kirsten Mayne
Telephone: (07) 3234 1260 (3406 8068)
Facsimile: (07) 3225 2629
Our Ref: 0243-0271-003

Associate Professor Alison Venn
Menzies Research Institute
Private Bag 23
HOBART TAS 7001

Dear Assoc Prof Venn

Your research proposal entitled "Exposure to high dose estrogens in adolescence: long term effects on mammographic breast density" was considered at the BreastScreen Queensland Monitoring, Research and Evaluation Sub-committee meeting held on Monday 5 December 2005.

The Sub-committee considered your request and support your application in principle. However BreastScreen Queensland will not allow original mammograms to be transported outside of the State to be digitised. Therefore, if you wish to use BreastScreen Queensland clients in your research you will need to make arrangements for original mammograms to be digitised in Brisbane. Any cost associated with the digitising of the mammograms must be met by the researchers.

In addition, valid, informed, written consent must be obtained from each BreastScreen Queensland client before access to identifying client information is permitted.

If you wish to discuss options for digitising original mammograms in Brisbane or you have any other questions, do not hesitate to contact Ms Kirsten Mayne, Senior Project Officer (Quality) on (07) 3234 1260.

Yours sincerely

Ms Jennifer Muller
Director, Cancer Screening Services Unit
16 / 12 / 2005

Office
8th Floor
Queensland Health Building
147-163 Charlotte Street
BRISBANE QLD 4000

Postal
Cancer Screening Services Unit
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BRISBANE QLD 4001

Phone
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UnitingCare Health
Human Research Ethics Committee

PO Box 499
Toowong Q 4066
Phone: 3232 7500 Facsimile: 3232 7109
Email: ethics@uchealth.com.au

Ms Helen Jordan
Menzies Research Institute
University of tasmnia
Privte Bag 23
HOBART TAS 7001

Document Submission and Approval Form

Correspondence: from Helen Jordan, University of Tasmania, dated 18th June 2007

Study Title: *Tall Girls Breast Density Study*

Investigator: Helen Jordan

Details of documents reviewed:

- Application for approval to access recent mammogram at The Wesley Breast Clinic

This application is approved.

Douglas Killer MBBS FRACP
Executive Officer

20/06/2007

The Uniting HealthCare Human Research Ethics Committee is constituted and operates in accordance with the National Health and Medical Research Council's Statement on Human Experimentation and Supplementary Notes

Telephone + 61 3 6226 7479
Facsimile + 61 3 6226 7148
Email Human.Ethics@utas.edu.au
www.research.utas.edu.au/human_ethics/

3 September 2007

AssocProf A Venn
Menzies Research Institute
University of Tasmania
Private Bag 23
Hobart Tas 7001

Dear AssocProf Venn

REF NO: **H0008334**
TITLE: **Adolescent exposure to hormone treatment for tall stature in girls: long terms effects on breast tissue**

Extension of data collection to include birth weight and length for both treated and untreated women

The Tasmanian Health and Medical Human Research Ethics Committee considered and approved the above documentation at its meeting on 3 September 2007.

All committees operating under the Human Research Ethics Committee (Tasmania) Network are registered and required to comply with the *National Statement on Ethical Conduct in Human Research* (NHMRC 2007).

Should you have any queries please do not hesitate to contact me on (03) 6226 2763.

Yours sincerely

Katherine Shaw
Ethics Officer, Health and Medical
On behalf of the Executive Officer
HREC (TAS) Network

Tasmania
Explore the possibilities

APPENDIX 15

Consent forms to obtain medical records

Menzies Research Institute

STUDY ID NUMBER: _ _ _ _ _ _

CONSENT FORM

Consent to obtain medical information for the:
Study of long-term health and psychosocial effects of hormone treatment to reduce the adult height of tall girls.

We would like to contact the doctor(s) who assessed and/or treated you for tall stature. We will, however, only do this with your permission. We are requesting access to your medical records so that we can confirm details about your height assessment and, if applicable, your treatment for tall stature. All the information we collect will be kept **confidential**. The information we collect will be **used solely for the purposes of this research study**.

Please provide us with the name and address(es) of the doctor or hospital who assessed or treated you for tall stature, and the year that you attended. If you are unsure of a doctor's name or address, please give us whatever information you do have.

(1) Dr or hospital_____Year attended _____
Address_____
_____ State_____Postcode_____

(2) Dr or hospital_____Year attended _____
Address_____
_____ State_____ Postcode____

(3) Dr or hospital_____Year attended _____
Address_____
_____ State_____ Postcode____

Please sign here to give your consent

I, _____ give permission for Dr Alison Venn from the Centre for the Study of Mothers' and Children's Health, La Trobe University, to contact my above named doctor(s), to verify information from my medical records regarding my assessment and/or treatment for tall stature.

Signed _____ Date _____

Maiden name if different from above _____

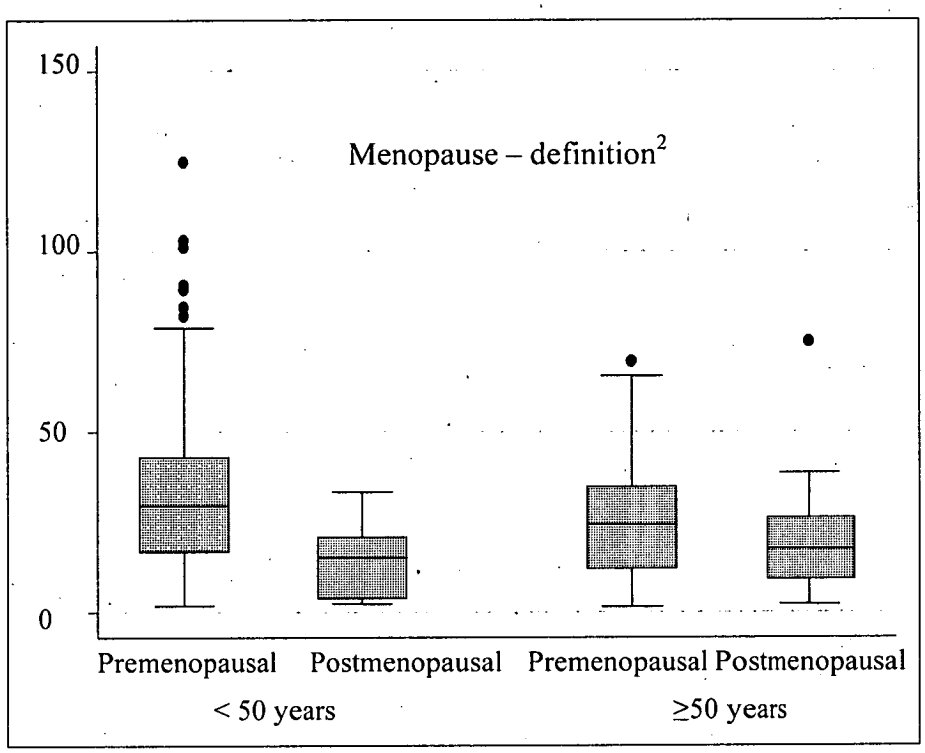
Thank you for this information

Appendix 15: Consent forms to obtain medical records

APPENDIX 16

Box-plots of dense area, percent density, non-dense area and total breast area by menopausal status (alternative definition*) and age category (<50 years, ≥ 50 years of age)

Figure A16.1: Box-plot of dense area (cm^2) by menopausal status and age category (<50 years, ≥50years).



* Postmenopausal if last period ≥ 52wks, and if HRT started before last period and current age was ≥55 years but women who had not had a period for ≥52 weeks because of hysterectomy (while retaining one or both ovaries), endometrial ablation, IUD, or hormone implants, were considered to be premenopausal unless they were ≥55 years of age.

Figure A16.2: Box-plot of percent mammographic density by menopausal status and age category (<50 years, ≥50years).

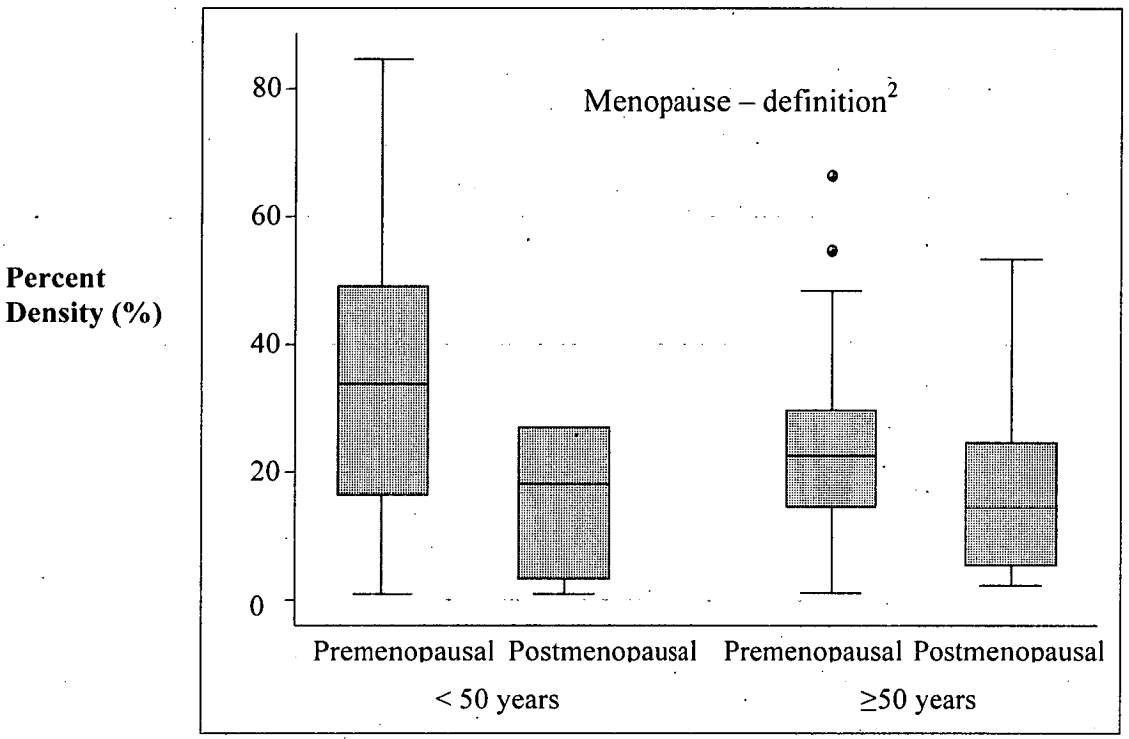


Fig A16.3 : Box-plot of total breast area (cm^2) by menopausal status and age category (<50 years, ≥50years).

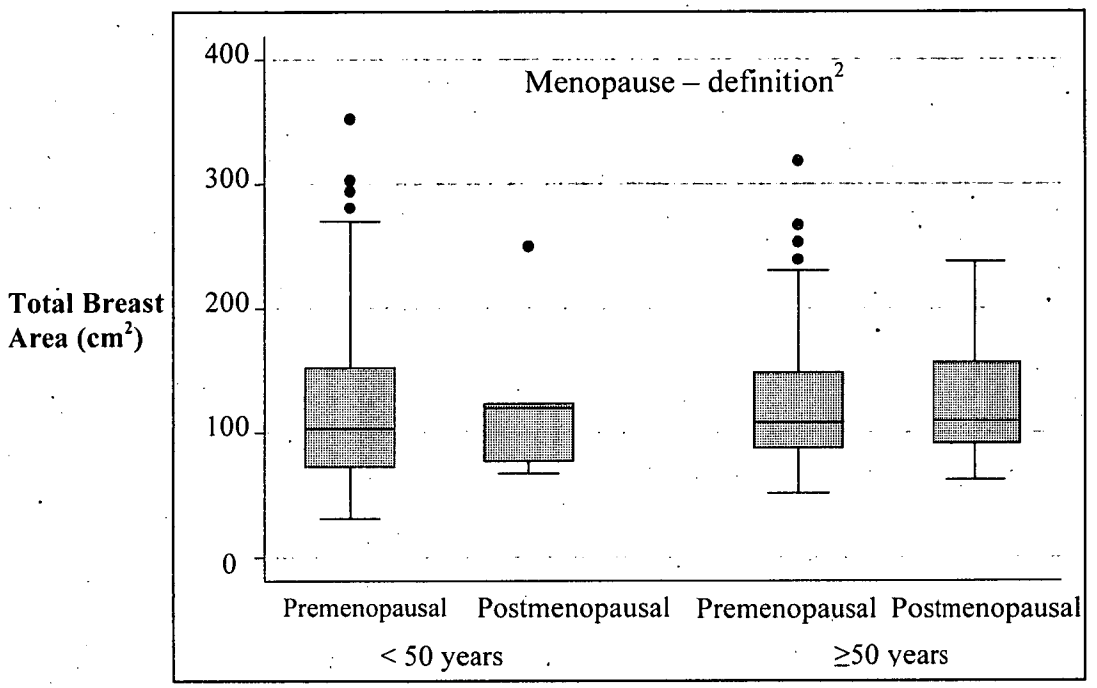
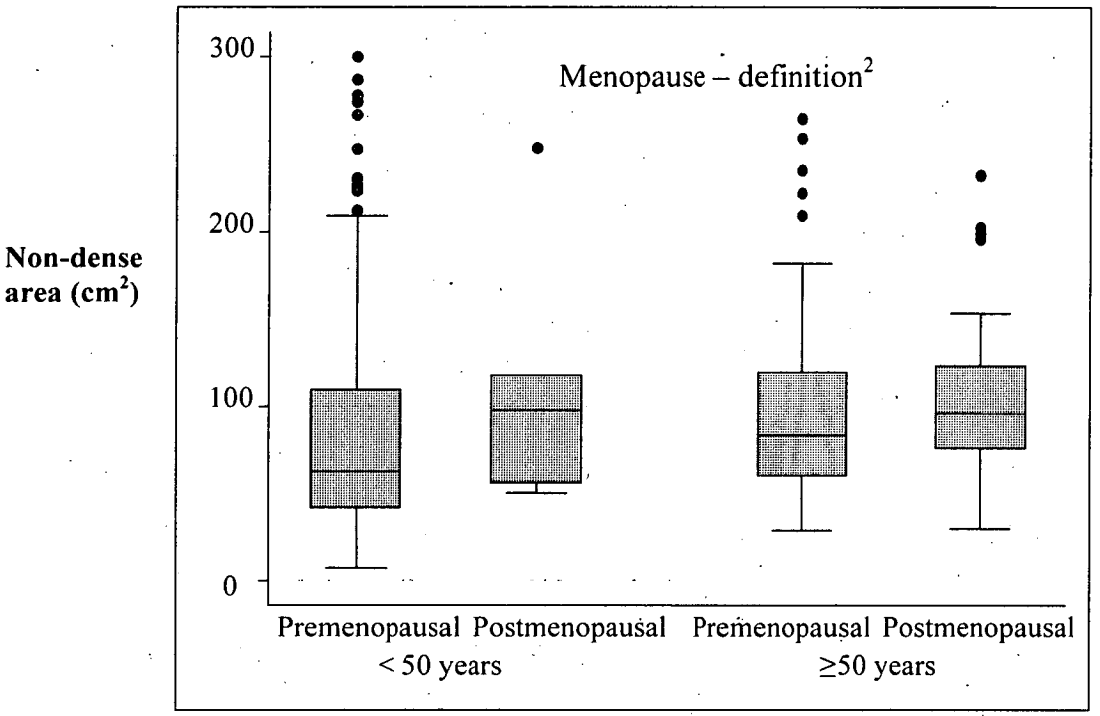


Figure A16.4: Box–plot of non-dense area (cm^2) by menopausal status and age category (<50 years, ≥50years).



APPENDIX 17

Post-estimation diagnostics for treatment effect on dense area

A number of diagnostic tests were performed on the regression model to verify that it fulfilled the assumptions of linearity and normality, and to identify and examine highly influential data points.

Collinearity

Collinearity between independent variables in regression analysis can cause imprecise and inflated differences in regression coefficients, and increase the standard error with the added consequence of reducing the test of significance[1].

A useful way to detect collinearity between independent variables in a regression is to examine the variance inflation factors (VIF) for each variable in the regression or the inverse (Tolerance factors)[1]. Individual VIF values greater than 10 or an overall mean VIF of >6 should be examined. As well, tolerance values (1/VIF) if small (e.g. <0.10) should be examined[1].

Variance inflation and tolerance factors for the independent variables in the regression of treatment effect on dense area, adjusted for age and BMI are presented in **Table A17.1.**

Table A17.1: Variance Inflation Factors and Tolerance factors (1/VIF) for the regression of treatment effect on dense area adjusted for age and BMI.

Independent Variable	**VIF**	**1/VIF**
Treatment	1.08	0.93
Age	1.07	0.94
BMI	1.02	0.98
Mean	**1.06**	**0.95**

The VIF and TIFs in **Table A17.1** suggest no issue with collinearity in the regression of treatment effect on dense area. To confirm these findings, the individual correlations between each of the independent variables is presented in **Table A17.2.**

Table A17.2: Correlation coefficients of each of the independent variables in the regression of treatment on dense area adjusted for age and BMI.

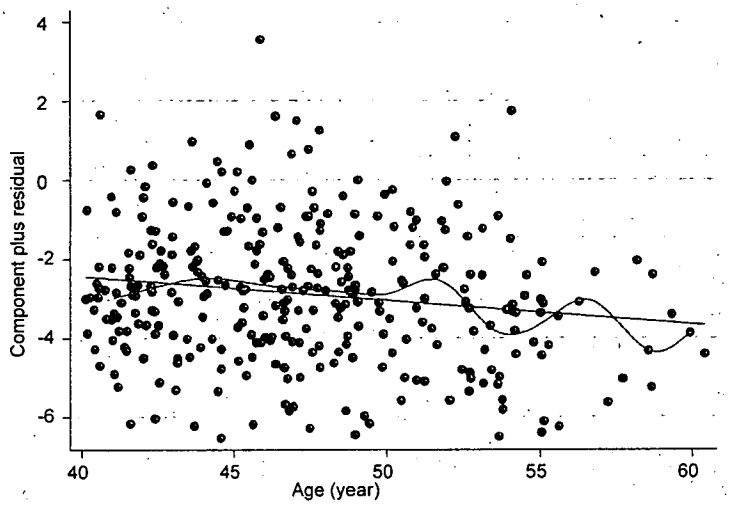
	Treatment	Age	BMI
Treatment	**1.00**		
Age	0.24	**1.00**	
BMI	-0.12	0.05	**1.00**

Functional Form of the Model

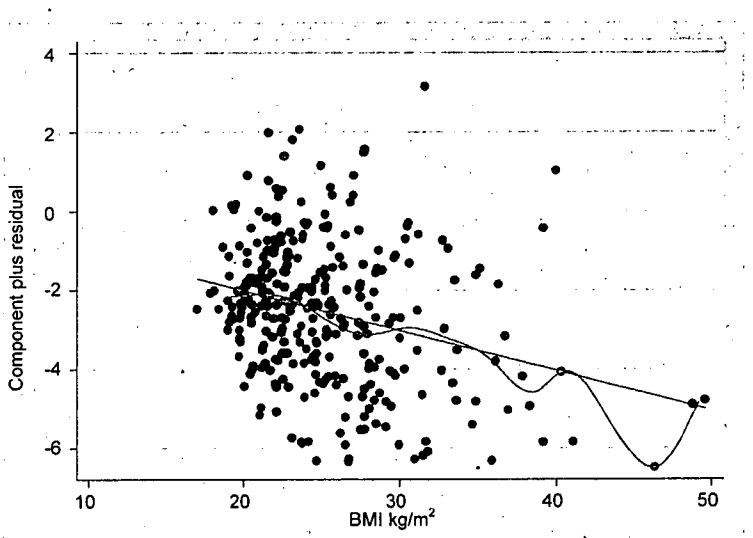
Each of the variables needs to demonstrate linearity so that the regression line through the coordinates has a slope equal to the estimated coefficient in the regression equation[2]. A plot that is useful for examining the functional form of the regression equation is the component-plus-residual plot[2]. Component-plus-residual plots for each of the continuous variables in the regression equation are presented in **Figures A17.1.**

Figures A17.1 Component plus Residual Plots of continuous variables a) age, and b) BMI in the regression of treatment effect on dense area (cm^2) (sqrt).

a) Age



b) BMI



The plots suggests no major issue of non-linearity in the regression line for either age or BMI, though BMI may require further investigation using a fractional polynomial model comparison test.

Fractional Polynomial Model Comparison

A Fractional Polynomial Model Comparison was undertaken of BMI and age and linear models were found to be suited for both (**See Tables A17.3** and **A17.4** below)

Table A17.2 Fractional polynomial model comparisons test for age in the regression of treatment effect on dense area adjusted for age and BMI.

Age	df	Deviance	Res. SD	Gain	P (term)	Powers
Not in model	0	1226.699	1.76984	--	--	
Linear	1	1219.190	1.75133	0.000	0.007	1
m = 1	2	1217.952	1.74783	1.237	0.270	3
m = 2	4	1214.105	1.73983	5.085	0.153	-2 -2

Table A17.3 Fractional polynomial model comparisons test for BMI in the regression of treatment effect on dense area adjusted for age and BMI.

BMI	df	Deviance	Res. SD	Gain	P(term)	Powers
Not in model	0	1244.628	1.82194	--	--	
Linear	1	1219.190	1.75133	0.000	0.000	1
m = 1	2	1219.190	1.75133	0.000	1.000	1
m = 2	4	1219.174	1.75416	0.016	0.992	.5 3

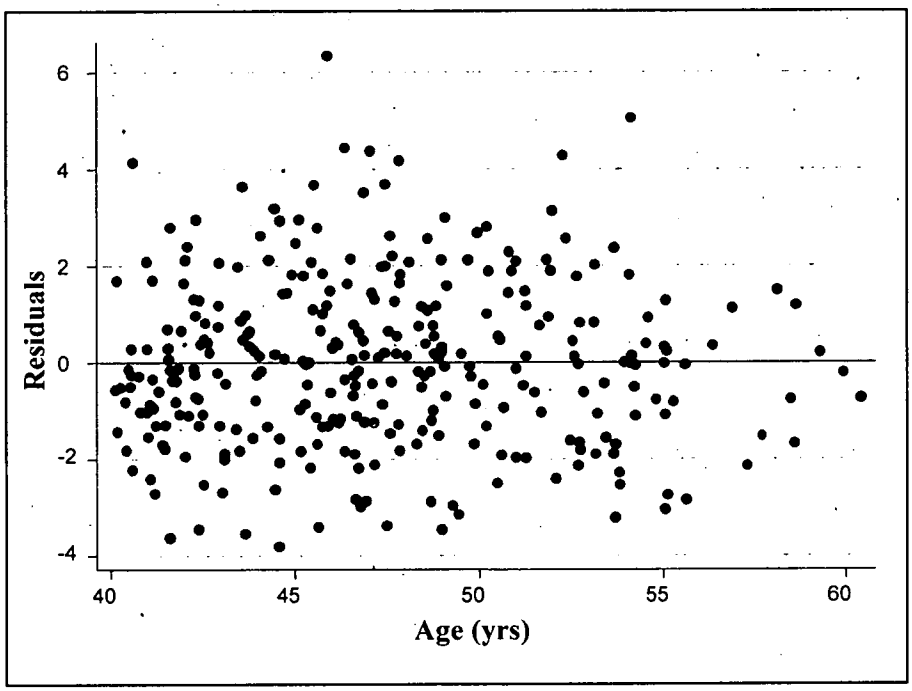
The "not in model" refers to the scenario where age or BMI is not affecting the outcome variable. The p-value indicates that the linear model is significantly different to 'not in model' for both age and BMI.

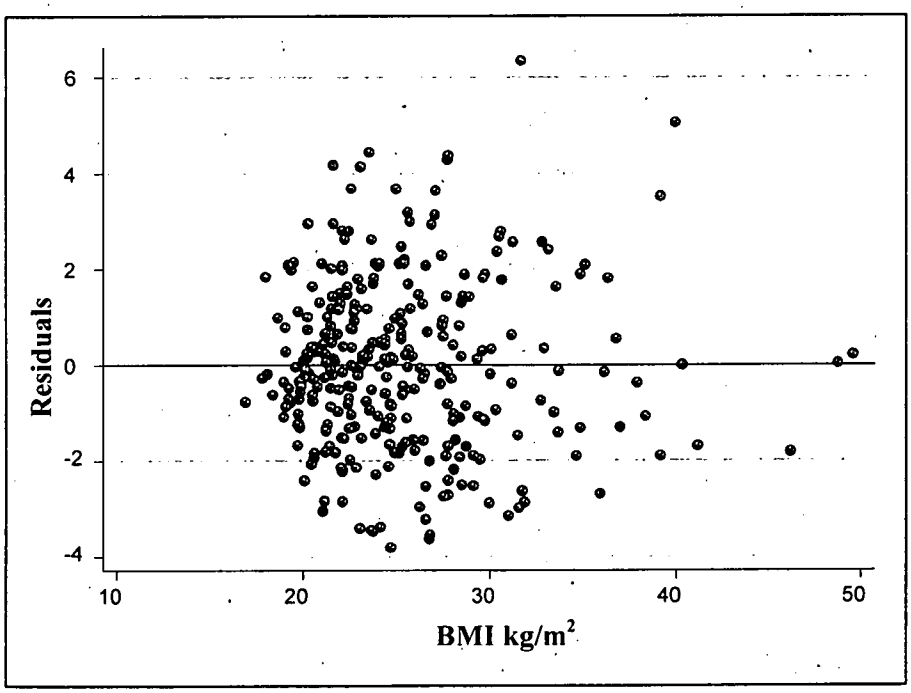
Residual vs Predictor Plots

Another assumption of linear regression is that variance in the residuals is random. Residual vs predictor or residual vs fitted plots provide a way to examine the distribution of the variance. If

the assumption of random variance is fulfilled, there should be no pattern in the plots[2]. For instance, the variation in the residuals should not change as the variable of interest changes[2]. Residual vs predictor plots for each of the continuous variables age and BMI are presented in **Fig. A17.2**

Figure A17.2. Residual vs predictor plots for age and BMI (kg/m^2)

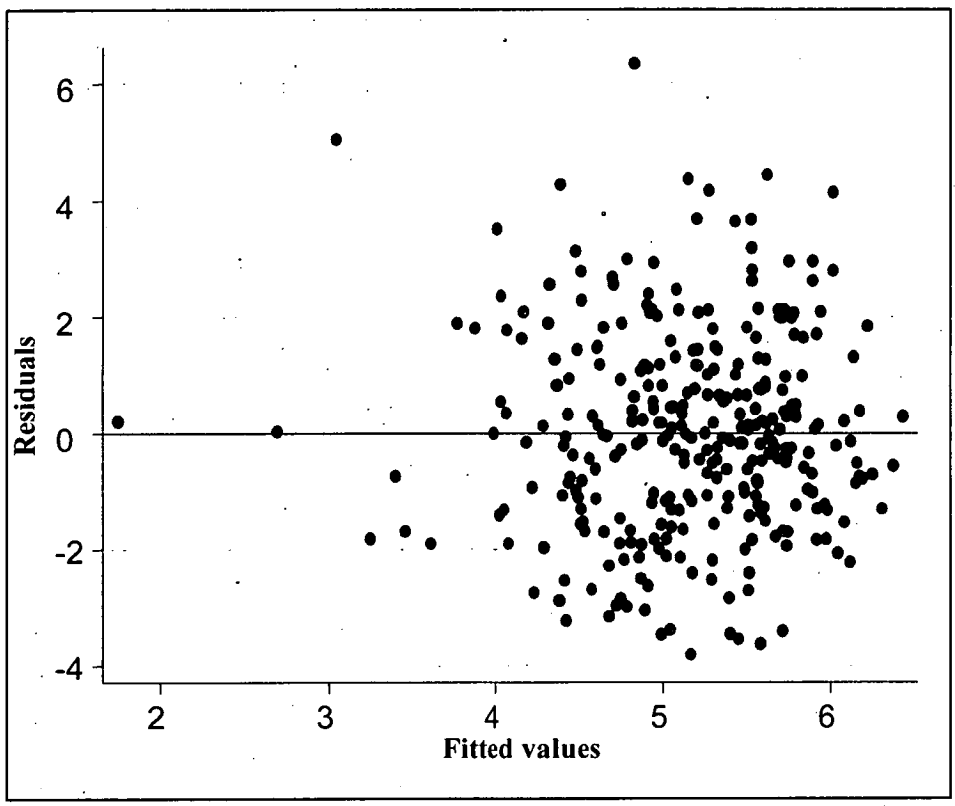




The assumption appears to be fulfilled for age and BMI. A few data points of high BMI present a potential issue, but these are too few to be conclusive. More data points in the higher BMI range might show a more consistent pattern in the variation about zero.

A residual vs fitted plot of the regression should also show no pattern to the residuals. A residual vs fitted plot is presented in **Figure A17.3**.

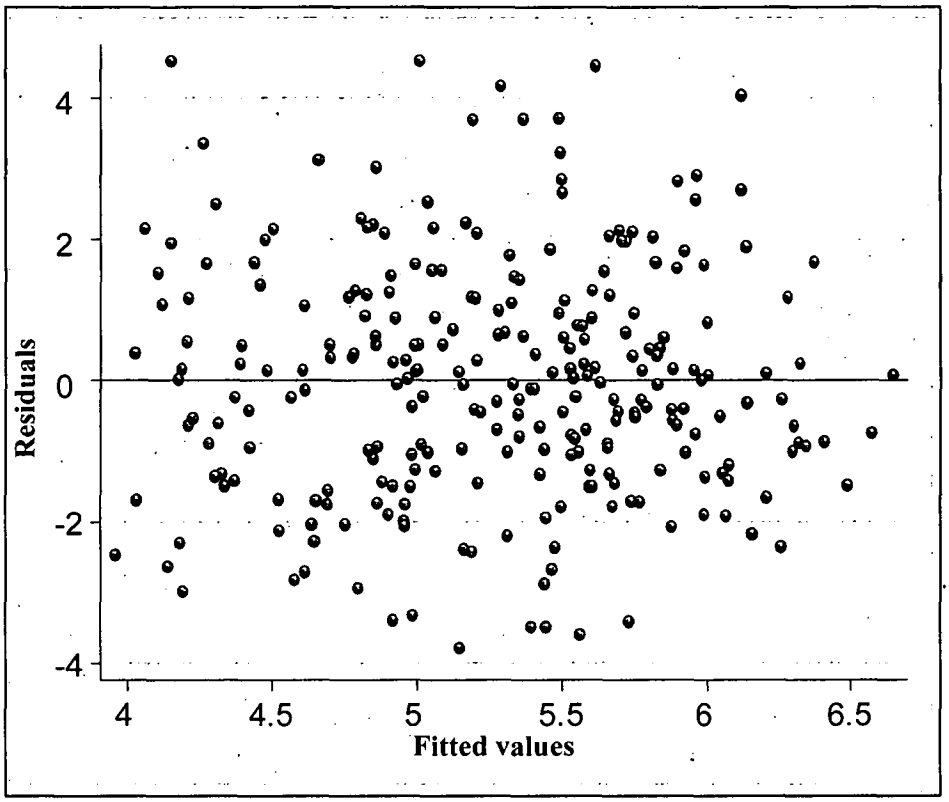
Figure A17.3: Residual vs fitted plot of the regression model.



The first seven points in the plot suggest a potential issue but again, there are too few data points here to suggest nonconstant variance or heteroskedasicity–an increasing or decreasing variation in the residuals plotted against the fitted values[2].

The observations with high BMI (>30) illustrated above in the residual vs predictor plots might be responsible for these patterns. Removing these values produces a more evenly distributed pattern of residuals against the fitted values (see **Figure A17.4**).

Figure A17.4: Residual vs Fitted plot (N=266) without the observations with BMI >30.



The regression coefficients for treatment effect on dense area did not significantly change when the observations of BMI >30 (n=43) were removed from the dataset (see **Table A17.4**).

Table A17.4: Regression results with and without BMI >30

	Coefficient (SE)	95% CI	*p* value
Regression (n=309)	-0.45 (0.21)	-0.86 to -0.04	0.032
Regression w/o values with BMI>30 (n=266)	-0.46 (0.21)	-0.88 to -0.04	0.035

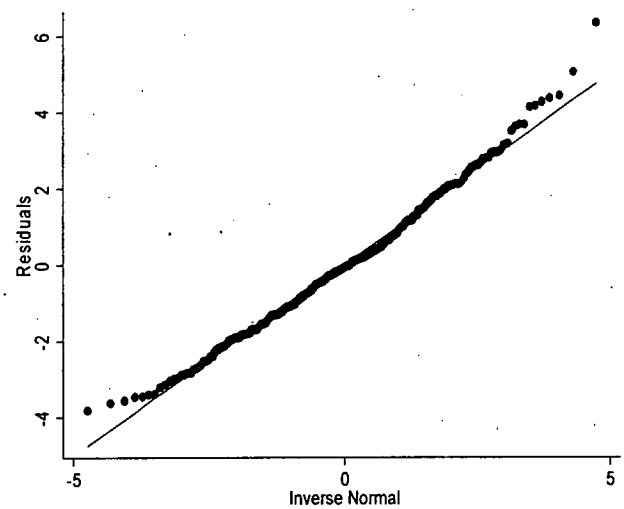
Heteroskedasticity

A Breusch-Pagan/Cook-Weisberg test for heteroskedasticity was performed and some degree of heteroskedasticity was suggested (p=0.035). To accommodate this, a robust regression was performed. The robust regression did not have any significant effect on the age and BMI adjusted regression coefficient, standard error or p-value for treatment effect on dense area [-0.45 (SE 0.20) (95% CI: -0.84 to -0.05; p=0.026)] compared with the non-robust regression [-0.45 (SE 0.21) (95% CI: -0.86 to -0.04; p=0.032)].

Test for Normality

Another assumption that needs to be fulfilled in linear regression is that the residuals are normally distributed. A plot of actual vs expected residuals is presented in **Figure A17.5**.

Figure A17.5: Actual residuals vs expected residuals.

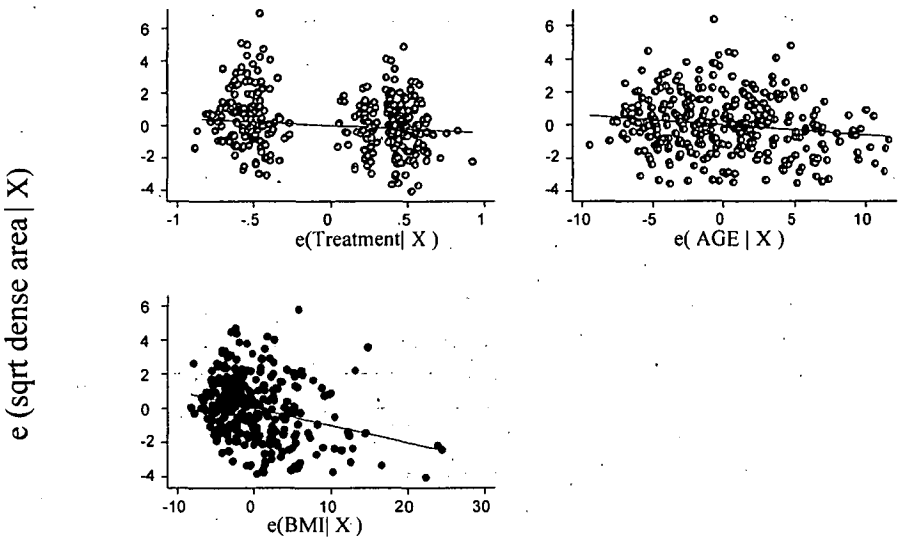


This plot suggests that the actual residuals meet the assumption of normality.

Test for outliers

Added variable plots (see **Figure A17.6**) allow for outliers, with extreme y-values, to be observed.

Figure A17.6: Added variable plot for treatment, age and BMI.

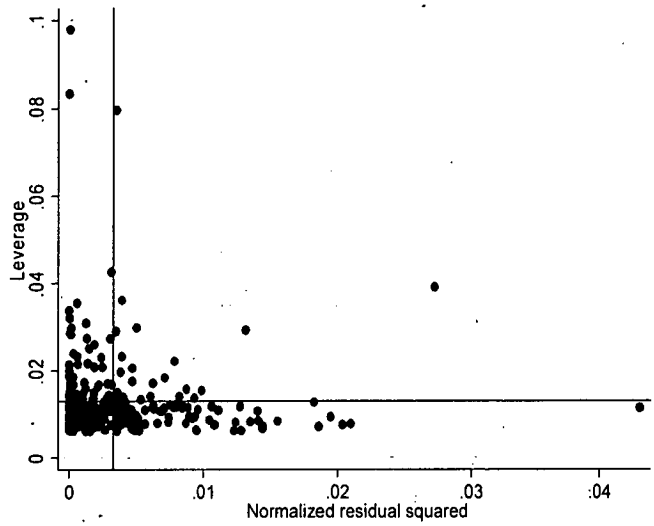


According to **Figure A17.6**, there appear to be a few potential outliers. Tests of influence were undertaken to assess the potential of these and other observations for influencing the results.

Tests of influence

A leverage vs squared residual plot (see **Figure A17.7**) can highlight potential influential observations. Points above the horizontal have higher than average leverage, while points to the right of the vertical line have higher than average residuals[2].

Figure A17.7. Leverage and squared residual plot for the regression of treatment effect on dense area adjusted for age and BMI.



If an observation has both a large residual and high leverage, that observation is potentially influential[3]. According to the leverage vs residual squared plot above **(Figure A17.7)**, a few observations could be considered to be influential. A more specific measure of the degree of influence is the Cooks D test, or Cook's distance[2].

Cooks Distance

Cook's D is one measure of influence of an observation. A Cooks distance test was performed. Seven points were found to have a Cooks d of >4/n (range 0.013-0.087). These were removed from the regression and the effect was observed to be minimal with the regression coefficient for treatment effect on dense area (sqrt) (See **Table A17.5**).

Table A17.5: Regression results with and without the points with Cooksd >=4/n. for treatment effect on dense area (cm^2) (sqrt).

	Coefficient (SE)	95% CI	*p* value
Regression (n=309)	-0.45 (0.21)	-0.86 to -0.04	0.032
Regression w/o values with Cooks d ≥ 0.04 (n=302)	-0.42 (0.20)	-0.81 to -0.03	0.036

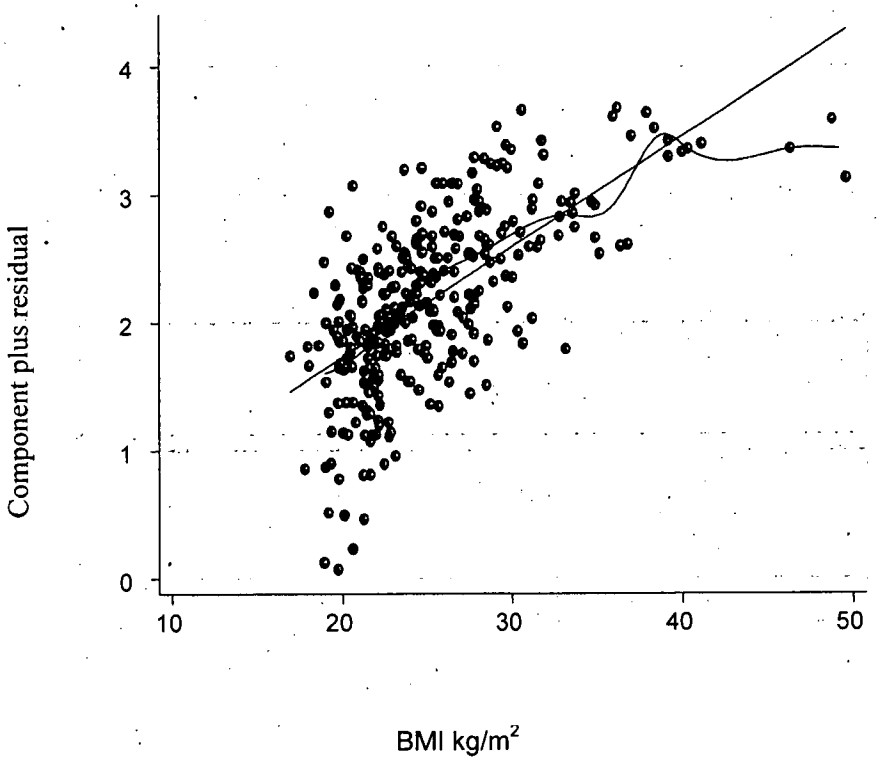
APPENDIX 18

Component plus residual plot for BMI in the regression for treatment effect on non-dense area

Appendix 18: Component plus residual plot for BMI in the regression for treatment effect on non-dense area.

Post-estimation diagnostics and sensitivity analysis was performed as for dense area and percent density. No influential points were found to significantly change the results. Removing digital images from the analysis did not affect the results. While the assumptions of linearity and normality were found to be fulfilled for the independent variables age and livebirths, this was not the case for BMI. While BMI and non-dense area are positively correlated, the relationship is curvilinear (the gradient is reduced at the larger end of the BMI scale) (See **Figure A18.1**).

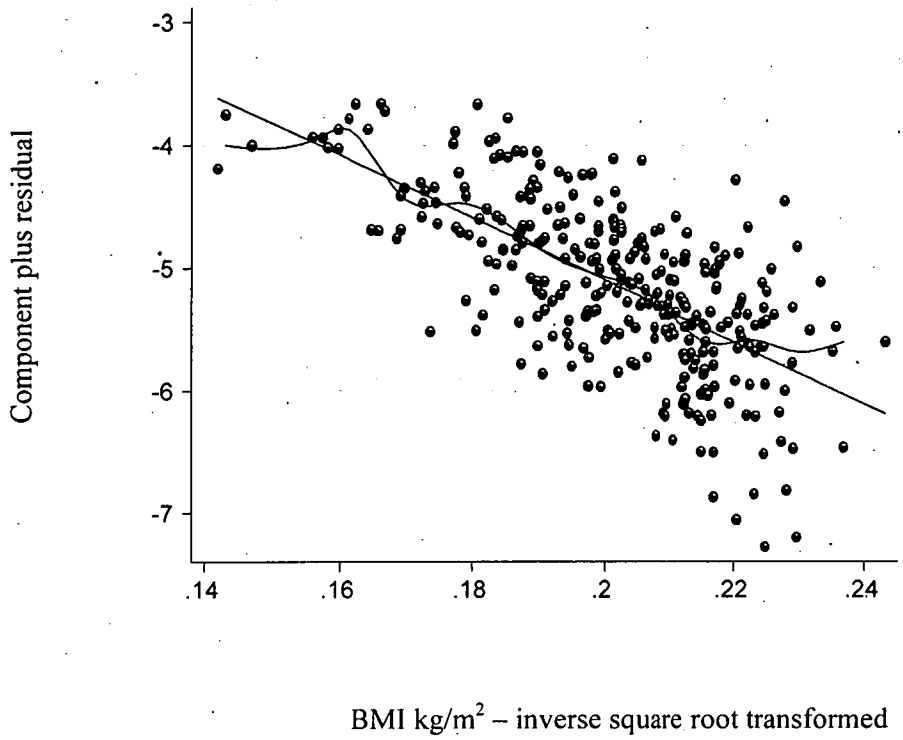
Figure A18.1 Component plus residual plot of BMI in the regression equation for non-dense area adjusted for age, BMI and livebirths.



For BMI adjusted analyses, where the response variable is non-dense area, an inverse square root transformation of BMI was carried out to meet the assumptions of linearity (See **Figure A18.2** below for the component plus residual plot of BMI after transformation. While the coefficient of the transformed BMI will now be negative this does not matter in the results as it is the coefficient for treatment that is of interest here.

Appendix 18: Component plus residual plot for BMI in the regression for treatment effect on non-dense area.

Figure A18.2 Component plus residual plot of BMI (inverse square root transformed) in the regression for non-dense area adjusted for age, BMI and livebirths.



APPENDIX 19

Ethics approvals to access data previously collected at first follow-up and again to extract additional information from the medical records (birthweight and birth-length)

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(TASMANIA)
NETWORK

UTAS

3 September 2007

AssocProf A Venn
Menzies Research Institute
University of Tasmania
Private Bag 23
Hobart Tas 7001

Dear AssocProf Venn

REF NO: **H0008334**
TITLE: **Adolescent exposure to hormone treatment for tall stature in girls: long terms effects on breast tissue**

Extension of data collection to include birth weight and length for both treated and untreated women

The Tasmanian Health and Medical Human Research Ethics Committee considered and approved the above documentation at its meeting on 3 September 2007.

All committees operating under the Human Research Ethics Committee (Tasmania) Network are registered and required to comply with the *National Statement on Ethical Conduct in Human Research* (NHMRC 2007).

Should you have any queries please do not hesitate to contact me on (03) 6226 2763.

Yours sincerely

Katherine Shaw
Ethics Officer, Health and Medical
On behalf of the Executive Officer
HREC (TAS) Network

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